50th ANNIVERSARY OF THE FOUNDING OF
THE AMERICAN SOCIETY OF TROPICAL MEDICINE

VOLUME 2 January 1953 NUMBER 1

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

Combining The American Journal of Tropical Medicine and The Journal of the National Malaria Society

OFFICIAL ORGAN OF
THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

PUBLISHED BIMONTHLY BY
THE WILLIAMS & WILKINS COMPANY
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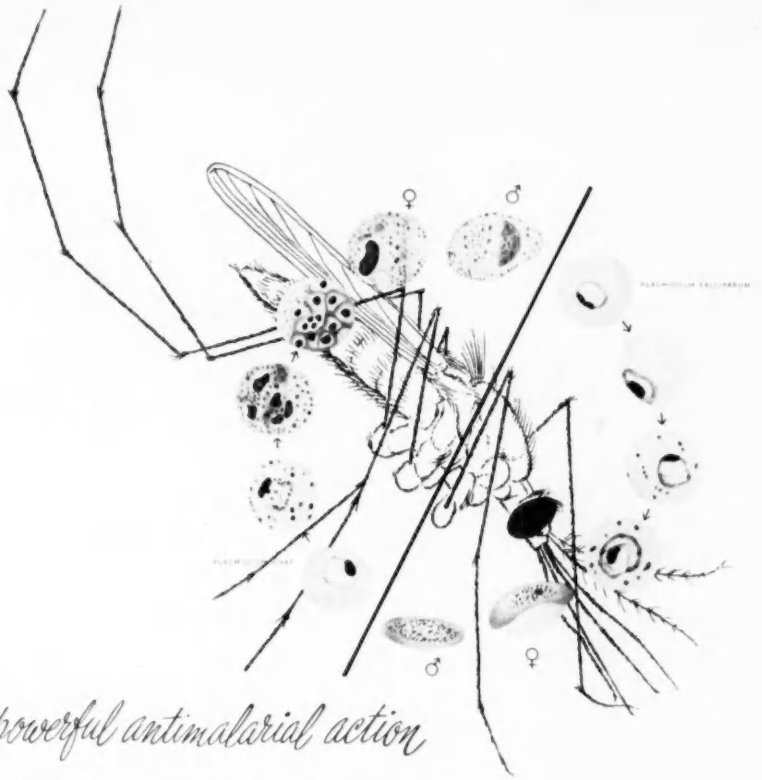

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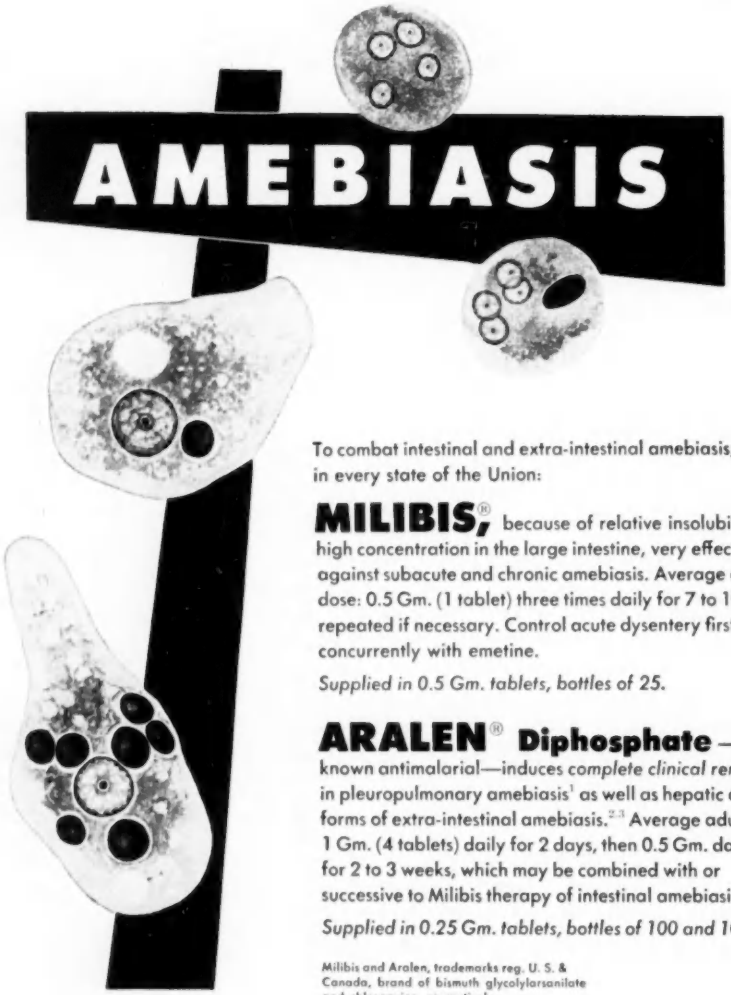

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1. Lindsay, A. E., Gossard, W. H., and Chapman, J. S.: *Dis. Chest*, 20:533, Nov., 1951.
2. Conan, N. J., Jr.: *Am. Jour. Med.*, 6:309, Mar., 1949.
3. Emmett, J.: *J.A.M.A.*, 141:22, Sept. 3, 1949.

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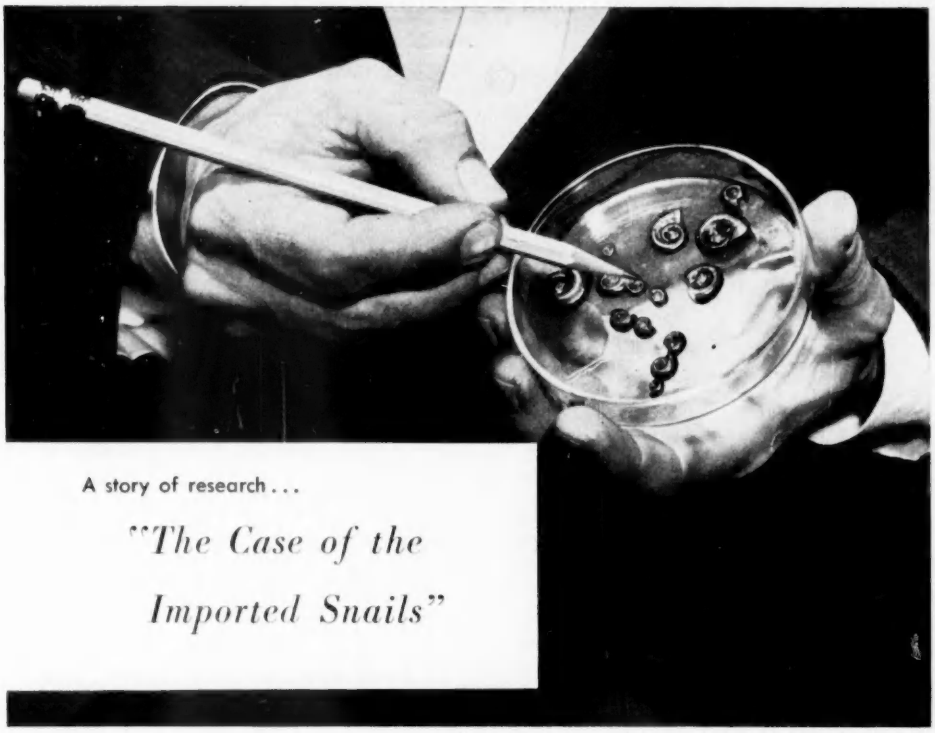

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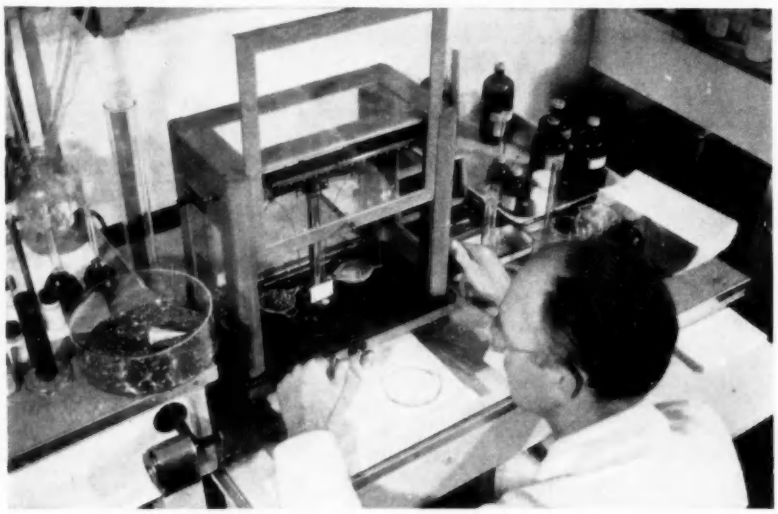

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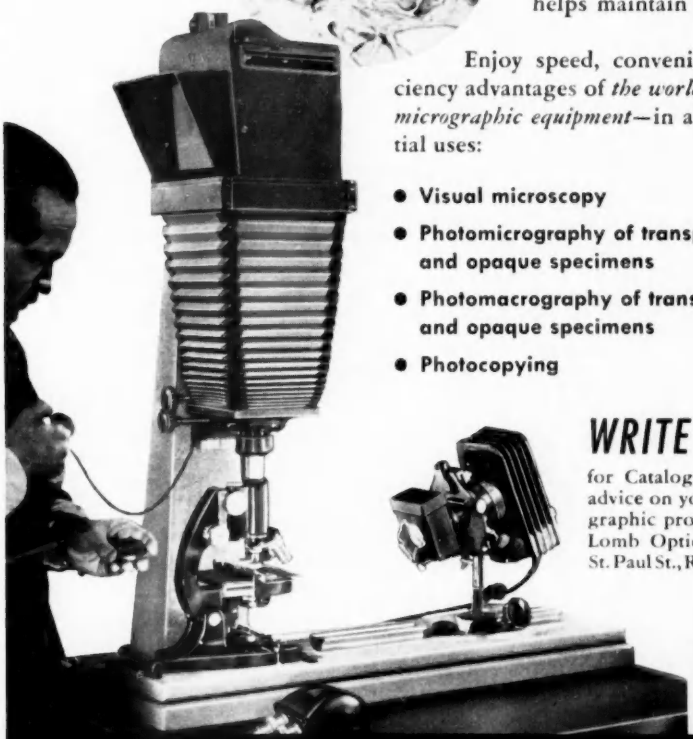

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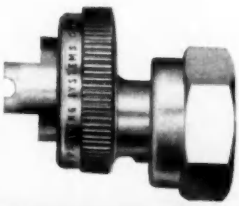

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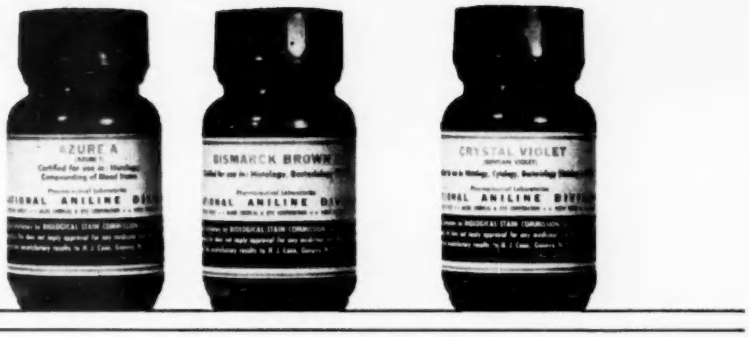

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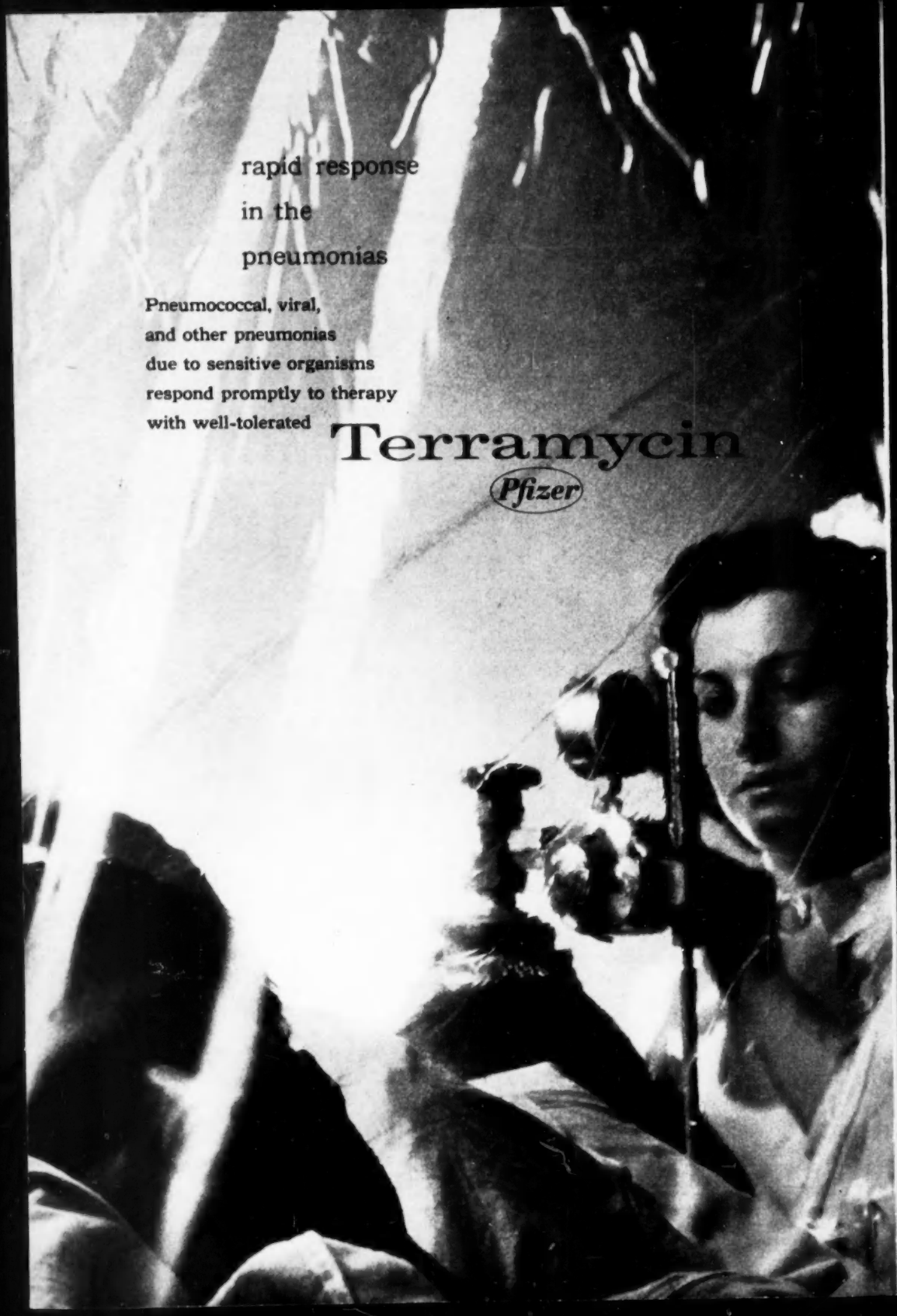
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MEDICAL MISSION TO THE YEMEN, SOUTHWEST ARABIA, 1951

I. Geomedical Observations[1]

ROBERT A. MOUNT[2]

U. S. Naval Medical Research Unit No. 3, Cairo, Egypt

Very little information has been published about the health of the people of the Yemen. This country, which lies in the southwest corner of the Arabian peninsula, has for many years remained rather completely isolated from the outside world. Only occasionally have scientific investigators penetrated its borders. The only published medical reports that have been found are those by Petrie (1939), Ganora (1930) and Sarnelli (1935). Petrie's report presents a brief general picture of the physical features and major diseases encountered in different geographic regions of the Yemen. Ganora described an outbreak of relapsing fever in San'a during the last three months of 1929. Sarnelli reported on the presence of schistosomiasis in the Yemen.

In response to a request from the Yemen Government a group of investigators from the U. S. Naval Medical Research Unit No. 3. (NAMRU-3) at Cairo, Egypt carried out a general survey of the prevailing sanitary and health conditions of three geographic regions of the Yemen: Hodeida, Ta'izz and San'a, during the period between January 6 and February 19, 1951. The survey team comprised an epidemiologist, R. A. Mount; an entomologist, K. L. Knight; a medical zoologist, H. Hoogstraal; a parasitologist, R. E. Kuntz; a bacteriological technician, J. R. Baranski; and a general technical assistant and interpreter, A. A. Salah.

The work of the mission was divided into two phases. One phase entailed the examination of representative samples of children and adults. Pertinent data relating to the individual's medical history were recorded and a physical examination was made. Persons examined contributed a sample of blood for serological purposes, and blood smears, peri-anal swabs and fecal and urine samples for parasitological study. Serum samples were kept frozen in a portable electric deep-freeze box. Blood smears were packed unstained in boxes for later examination. A bacteriological culture for enteric pathogens was made from each fecal sample which was then preserved in vials containing polyvinyl alcohol (PVA) fixative and glycerinated formalin respectively (Kuntz *et al.* 1953). Urine samples and anal swabs were examined for helminth ova (Kuntz *et al.* 1953). Sera,

[1] The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

[2] Commander, MC, USN, Department of Virology, Naval Medical Field Research Laboratory, Camp Lejeune, N. C.

Grateful acknowledgement is made to the other members of the mission without whose assistance this part of the survey could not have been accomplished. The author is particularly endebted to Abdel Aziz Salah Effendi for invaluable technical assistance during physical examinations and the collection of historical data relating to disease incidence.

blood smears, cultures and fecal specimens were brought back to NAMRU-3 for study.

The other phase of the work dealt with the inspection of three different representative geographic areas of the Yemen and with the collection and study of arthropods and vertebrates. Several hundred wild and domestic rodents were collected and examined for external and internal parasites and for gross evidence of disease. Blood and tissue smears were taken for later examination at Cairo.

During the period of seven weeks spent in the Yemen, approximately 1000 people were examined. Of this number, 451 were selected as representative samples of the people residing in the three geographic regions studied. The remainder were obviously ill patients and were excluded from the general population sampling.

GEOGRAPHY AND CLIMATE

The independent state of Yemen lies in the southwest corner of the Arabian peninsula. It is bounded on the north by the state of Asir, Saudi Arabia, on the west by the Red Sea, on the south by the West Aden Protectorate, and on the east by the great Arabian desert. From the coastal belt along the Red Sea, termed the Tihama, which varies from about 20 miles to more than 100 miles in width, the land rises in steps to the central highlands. These extend north and south through the central region of the Yemen and range from about 6500 to nearly 11,000 feet in height. Toward the east the land drops to 3000 feet to form a large area of rolling country which finally merges with the Arabian desert.

Although no official census has been taken, the population of the Yemen has been variously estimated to number between three and seven million. The people are predominantly of Arabic descent.

The Yemen is divided into four geographic regions: the Tihama; the foothills and middle heights; the highlands; and the rolling tableland to the east. The Tihama, or coastal plain, ranges from sea level to about 600 feet. It is a hot, arid region and, for the most part, is relatively barren, having wide stretches of desert sand between the occasional oases and cultivated areas. It is estimated that less than one-tenth of the total population of the Yemen lives in this coastal plain belt.

The foothills and middle heights comprise an irregular belt of greater area than that of the coastal plain, and range in elevation from 600 to 4500 feet (Fig. 1). From the foothills bordering the Tihama inland, the terrain is broken by numerous deep valleys leading upward stepwise to the high table lands. Throughout this belt subsurface water is plentiful with numerous artesian hot or cold water springs and small streams. Elaborate terracing of mountain slopes with careful attention to levels and land contours has made large areas arable. Much of the green produce of the country comes from the middle heights. These products consist principally of Mocha coffee, citrus fruits, apples, bananas, grapes, dates, walnuts, vegetables, dhurra and qat.[3] The meteorological data recorded by Tof-

[3] Qat (*Catha edulis*) is a small slender shrub or tree generally not exceeding ten feet in height. It grows along the sides of steep valleys between 4500 and 8300 feet elevation. The cultivation of this plant is associated with a social practice of chewing the tender leaves, a habit widespread throughout this section of the Arabian Peninsula.

folon (1946) indicates that the climate of the middle heights is remarkably pleasant throughout the year. A summary of his records for 1945 is given in Table 1.

The highlands comprise a wide belt of tablelands broken by mountain peaks and ranges. For the most part these tablelands are relatively free from the luxuriant natural vegetation seen at lower altitudes. Where the high plateaus are

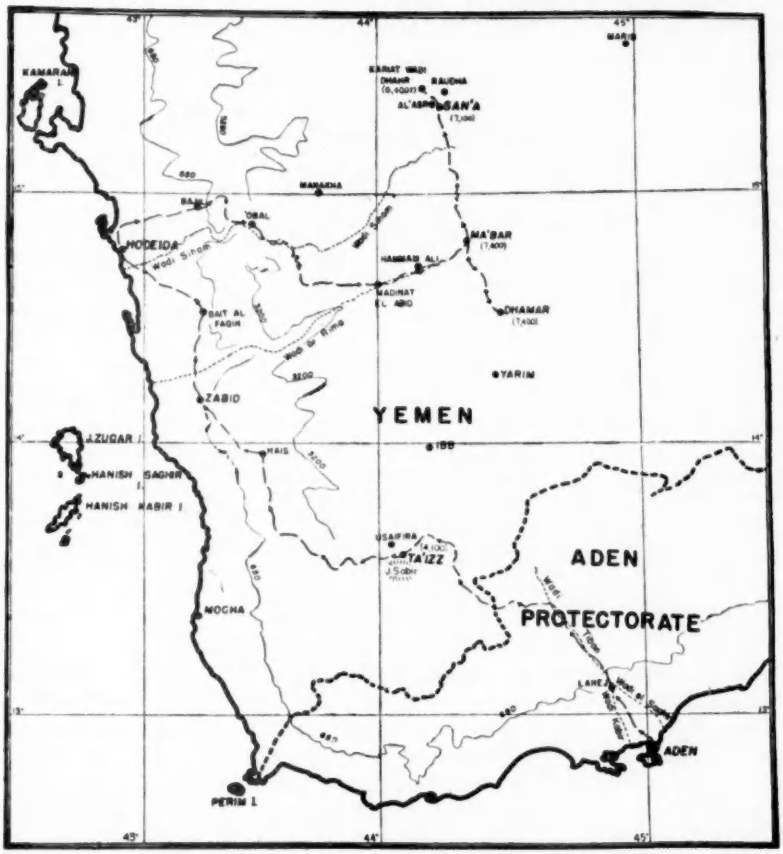

FIG. 1. Map of the Yemen showing the route (→) taken by the Naval Medical Mission, 1951.

broken by peaks and valleys, fertile fields are found in their shelter. There are some perennial streams in the highlands but, as in the lower areas, wells which descend from 40 to 200 feet to reach water level are used to utilize the abundance of subsurface water. Water is drawn by hand or by animals and provides for the mosques, for drinking, and for irrigation of some of the fields.

The climate at San'a is somewhat similar to that of Asmara, Eritrea, which

lies almost directly across the Red Sea at a slightly greater altitude. Petrie (1939) reported a mean temperature at San'a in June of 68°F. (20°C.) and in November, the coldest month, a mean temperature of 59°F. (15°C.). Both spring and autumn rains occur at San'a. Petrie (1939) reported a total precipitation of 350 to 375 mm. for the year.

PUBLIC HEALTH

Organization. The Yemen has a Ministry of Health which is staffed by non-medical personnel without specialized training or experience in preventive medicine.

TABLE 1

Meteorological data for Ta'izz for the year 1945*

MONTH	TEMPERATURE		HUMIDITY†	RAINFALL IN MILLIMETERS	WIND DIRECTION FROM (PER CENT)	
	Max.	Min.			East	West
January	24.8	13.7	65.7	0.5	40.0	15.0
February	25.4	15.8	65.1	0.8	44.0	28.5
March	27.2	18.2	43.0	11.2	59.5	20.3
April	28.2	18.7	50.5	0.0	54.0	31.0
May‡						
June	29.8	20.9	44.7	23.5	6.0	58.0
July	29.4	22.0	44.5	45.0	2.0	72.0
August	28.5	19.6	54.9	61.7	5.4	57.0
September	28.6	20.2	53.6	33.2	6.7	23.3
October	27.3	16.8	53.0	95.2	41.8	26.2
November	25.6	15.6	60.5	1.8	45.6	23.4
December	24.7	14.8	62.3	35.4	28.3	33.2

* This data was recorded at Ta'izz, in the middle heights, at an elevation of 4,400 feet by Dr. C. Toffolon, a resident Italian physician.

† Average based on three readings per day.

‡ Not recorded.

Water supplies. Water for drinking is obtained either from open wells or from fresh water streams. At Ta'izz, the water supply is brought down from Jebel Sabir to the public reservoirs in the town through open conduits. The reservoirs are of two types, those which are used to store drinking water and are partially covered, and open reservoirs with water for animals, irrigation and for washing purposes. There is no piped distribution system. In the interior of the dwellings the water is usually stored in the kitchen, toilet and bathroom. Storage is in earthen pots of varying capacities which may be open or imperfectly covered.

Sewage and garbage disposal. There is no organized means of garbage or sewage disposal in the cities visited. In homes, urine and feces are separated by the simple device of depositing the former in an open trough in the floor of the washroom and the latter in a central collection chamber on the ground floor which receives the human excreta from the floors above. The urine flows directly outside the building down a smooth plastered groove on the front of the building onto the street below.

Arthropods and snails of medical importance. **Mosquitoes.** *Anopheles dthali* Patton, *A. gambiae* Giles, and *A. adenensis* Christophers have been reported from the Yemen (Buxton, 1944; De Meillon, 1947). *A. turkhudi* Liston, *A. cinereus* Theobald, and *A. sergenti* Theobald have also been taken in the Yemen (Merucci, 1951).

The mosquito collection made by Knight during the course of the present survey contained the following nine species of anophelines: *Anopheles adenensis* Christophers, *A. cinereus* Theobald, *A. dthali* Patton, *A. fluviatilis* James (identification of this species remains questionable), *A. gambiae* Giles, *A. pharoensis* Theobald, *A. pretoriensis* Theobald, *A. rupicolus* Lewis, and *A. sergenti* Theobald. No specimens of *A. turkhudi* were taken.

Eighteen species of culicine mosquitoes were collected. These are: *Culiseta longiareolata* (Macquart), *Aedes aegypti* Linnaeus, *Aedes* (*Aedimorphus*) sp. (near *A. natronius*), *Culex tigripes* Grandpré and Charmoy, *Culex* (*Neoculex*) sp. (near *C. salisburiensis*), *Culex* (*Neoculex*) sp. (near *seyrigi*), *C. nebulosus* Theobald, *C. desens* Theobald, *C. ethiopicus* Edwards, *C. fatigans* Wiedemann, *C. laticinctus* Edwards, *C. pipiens* Linnaeus, *C. simpsoni* Theobald, *C. sinaiticus* Kirkpatrick, *C. sitiens* Wiedemann, *C. theileri* Theobald, *C. tritaeniorhynchus* Giles, and *C.* sp. (near *laticinctus*).

Lice. *Pediculus corporis* and *Phthirius pubis* were found rather generally spread throughout the country.

Flies. Muscid flies were numerous in the towns of the Tihama where the temperature is favorable for breeding throughout the year. The densities of these flies were considerably lower in the middle heights and in the highlands where the temperature was still too low for the extensive fly breeding which is stated to occur in these locations during the summer months (Toffolon, 1951). Sandflies, (*Phlebotomus* spp.) were caught outside of the laboratories at Ta'izz as they attempted to bite volunteers who were stationed in a courtyard surrounding the building.

Ticks. Because camels, goats, cattle, horses, donkeys and mules are so numerous in the Yemen, ticks abound in astonishing numbers. Hoogstraal found *Hyalomma* ticks, representing at least four species, particularly prevalent. Hoogstraaal found the common dog tick, *Rhipicephalus s. sanguineus*, widespread throughout the Yemen. In the middle altitudes of the Yemen the East African tick, *Amblyomma variegatum*, was found in some numbers. Ticks of the family Argasidae were numerous in the three regions visited by the mission. A few specimens of *Argas* were taken but there was no indication that they were ever present in sufficient numbers to constitute a public health problem. *Ornithodorus savignyi* was found to be widespread in the Yemen. Although *O. moubata* has been reported for the Yemen (Petrie, 1939), no specimens were recognized in the present survey.

Mites. Numerous specimens of mites were collected from wild rodents and small game. Radford (1952) reports the presence of the mite *Dermonyssus muris* (Hirst, 1913) and six new species of Trombiculid mites among the collection.

Fleas. Domestic rats and mice, which were extremely abundant in all towns

and cities visited, often harbored large numbers of fleas (Sanborn and Hoogstraal, 1952). *Xenopsylla cheopis* was common on house rats in all Yemen towns and cities visited by the mission. The taxonomic report on the specimens collected is in preparation (Traub).

Mollusks. Approximately 85 collections of aquatic and terrestrial mollusks were taken in the Yemen. The collection included *Biomphalaria boissyi arabica* and *Bulinus contortus*, vectors of *Schistosoma mansoni* and *S. haematobium* respectively (Kuntz, 1952; Bequaert). The former species was taken in Ta'izz and the latter near San'a, at Wadi Dhahr.

MEDICAL FACILITIES

Hospitals. There are three hospitals in the Yemen, one at Ta'izz of approximately 100 beds, one of about the same bed capacity in Hodeida, and a larger one in San'a with an estimated 250 bed capacity. The hospital equipment was considered to be inadequate.

Medical personnel. At the time there were only four physicians practising in the Yemen. There were apparently no trained nurses. Three physicians were in Ta'izz and the other in San'a. Four Yemeni doctors are reported for the country. These have received approximately two years training in colonial medical practice in Italy. In addition, there are a number of Yemeni midwives who have received no formal training in nursing or obstetrics.

DISEASES

Diseases spread through discharges from the intestinal tract

Toffolon (1951) states that dysenteries, bacillary and amebic, as well as typhoid fever occur throughout the Yemen. Native midwives believe that acute dysenteries are the commonest cause of death in infants and young children. The results of stool cultures and serological examinations indicate that both *Shigella* and *Salmonella* infections are very prevalent in the Hodeida, Ta'izz and San'a districts (Mount and Baranski, 1953). Kuntz, *et al.* (1953) found the incidence of *E. histolytica* infections high in Ta'izz, Hodeida and the San'a-Ma'bar districts, over 30 per cent of 222 single fecal samples being positive for this intestinal protozoan.

Helminthiases. The results of the present survey indicate that hookworn infection is not a public health problem in the 3 regions of the Yemen visited. Merucci (1951) states that two individuals with hookworm infections visited his clinic at Hodeida in 1940. Both individuals came from southern Yemen. However, he states that subsequent surveys in Hodeida, Ta'izz and San'a failed to reveal any further evidence of this infection in the Yemen. Kuntz *et al.* (1953) found no hookworm ova in 496 preserved fecal specimens examined. Only 4 of the 496 single fecal specimens examined were positive for tapeworm (*Taenia saginata*). These were taken in the highlands near Ma'bar, where only 26 specimens were obtained.

Kuntz *et al.* (1953) found infections with whipworm (*Trichuris trichiura*) and

pinworm (*Enterobius vermicularis*) in all three of the geographic regions visited, whipworm ova being recovered from 9.5 per cent of the stools examined and pinworm ova from 12.7 per cent of the anal swabs.

Both *Schistosoma mansoni* and *S. hematobium* were found in the Yemen (Kuntz, 1952), thus confirming an earlier report by Sarnelli (1935) that both species do occur in the southern part of the Arabian Peninsula. *S. mansoni* and *S. haematobium* were found in 56 and 10 per cent respectively of stool and urine specimens taken from persons living at Ta'izz. The rates of infection for both species were much lower at Hodeida and no *S. haematobium* was found in specimens collected from the highland plateau of San'a. The high percentage of infection recorded for the inhabitants of Ta'izz, especially with *S. mansoni*, indicated that a highly efficient transmission cycle was operating either in the city itself or in the adjacent countryside close to Ta'izz. A survey of the limited

TABLE 2

Percentage distribution of pertinent history and physical findings in two age groups in the Yemen

	HODEIDA		TA'IZZ		SAN'A		TOTALS
AGES IN YEARS	Under 21	Over 21	Under 21	Over 21	Under 21	Over 21	
Number examined	129	44	76	131	54	17	451
History—% with:							
Cough	39.6	22.7	46.0	45.8	42.6	35.3	41.0
Blood in urine	3.1	4.5	18.4	13.7	9.3	0	9.5
Chills & fever	80.0	81.8	88.3	71.0	42.6	52.8	72.7
Epigastric pain	23.6	41.0	44.7	42.7	57.4	52.8	41.1
Chews qat	24.0	66.0	50.0	76.4	64.9	82.4	54.8
Physical—% with:							
Râles	10.7	15.9	21.0	22.1	5.6	5.9	15.5
Enlarged liver	6.9	9.1	38.2	3.8	1.8	0	10.6
Enlarged spleen	14.7	25.0	60.6	16.8	3.7	0	22.2

natural water catchments showed that planorbid snails were present in rather small numbers. None, however, were found to be infected when crushed or examined for emerging cercariae. A search of Ta'izz mosque basins used for ritualistic ablutions revealed that approximately 35 per cent of 75 *Biomphalaria boissyi arabica* from one mosque were infected with *Schistosoma mansoni* (Kuntz, 1952). Several more snails with early developmental stages of schistosome cercariae were found in a second mosque in Ta'izz.

Snail vectors were also found at Wadi Dhahr, near San'a, where *Bulinus contortus* was discovered in the ablution pools of mosques. However, none of the snails examined were found to be infected. This snail is also found in open storage cisterns frequently used by man and animals in the San'a area.

With regard to schistosomiasis it should be noted that 38.2 per cent of 76 persons under 21 years of age examined at Ta'izz possessed an enlarged liver. In the same group 60.6 per cent had demonstrable splenic enlargement (Table 2).

Diseases spread chiefly through the respiratory tract

Pulmonary tuberculosis is widespread throughout the country, and is said to account for a large percentage of the total morbidity and mortality of the people (Toffolon, 1951). Resident Italian physicians state that it is particularly prevalent among women and children. It may be observed in Table 2 that 41 per cent of 451 people physically examined complained of a persistent cough, and 15.5 per cent of the total number had râles in one or both lung fields.

Pneumonia, clinically lobar, has been reported to occur frequently in the middle heights and in the highlands, and to a lesser extent in the Tihama (Petrie, 1939). Several cases with lobar consolidation were seen in both Ta'izz and San'a during the present survey.

Smallpox epidemics were said to be fairly common until 12 to 15 years ago, particularly in the middle heights and the highlands (Petrie, 1939). About this time the Italian physicians came to the Yemen and shortly after their arrival a program of widespread vaccination against smallpox was begun. Petrie saw no cases of smallpox during his two years in the San'a district. No cases of smallpox were seen during our stay in the Yemen, although pockmarked faces were occasionally seen throughout the country.

Both *anterior poliomyelitis* and *meningococcic meningitis* have been reported for the Yemen (Petrie, 1939). No incidence figures are available.

Diseases spread chiefly by contact

Treponematosis. Both *syphilis* and *bejel* have been reported for the Yemen (Petrie, 1939). Syphilis was said by the resident physicians to be very prevalent throughout the country (Toffolon, 1951). In this regard, well over half of the people from whom serum samples were taken were found to have positive serology, based on a presumptive and a standard Kahn test (Mount and Baranski, 1953). However, since bejel is reported to be endemic in the Yemen (Petrie, 1939), it is not possible to know how great a percentage of the positive Kahns is of luetic origin. No recognized cases of bejel were encountered in the present survey.

Gonorrhea was said by resident physicians to be extremely prevalent throughout the country, no class being exempt. Actual figures could not be obtained.

Diseases of the skin. *Scabies* is very prevalent among the children of the laboring classes of the three regions visited. *Furunculosis* was frequently seen. *Tinea* infections and *epidermophytosis* are common. Several cases of *vitiligo* were seen during the survey. A progressive ulcerative lesion, termed *Yemen ulcer*, occurring over the lower tibial surface of the leg or on the ankles, is widespread. The etiology of these ulcers has not been determined. *Madura foot* or pseudomycosis is stated to occur in both the middle heights and the highlands (Merucci, 1951).

Diseases of the eyes. Blindness of one or both eyes and ophthalmic infections were commonly seen in all three geographic regions visited in the Yemen. In a survey of school children in the San'a district, Petrie (1939) found that 90 per cent of the Arab children had *trachoma*. *Gonorrheal conjunctivitis* is said to be widespread (Toffolon, 1951).

Tetanus is reported to occur fairly frequently in the Tihama, but to be rare in the mountainous districts (Petrie, 1939).

Leprosy of the nodular, anaesthetic and mixed types occurs. Petrie (1939) is of the opinion that it is predominately a disease of the middle heights, in the region between Ibb and Zabid. No data regarding the prevalence of this disease could be obtained. Segregation is not practiced. Cases of the mixed type were seen in all three geographic regions visited.

Brucellosis has not been reported for the Yemen. The resident physicians stated that this disease possibly occurs infrequently. The results of *Brucella* agglutination titrations of 261 sera collected suggest that this disease may be of fairly common occurrence in the two regions surveyed for this disease, Ta'izz and San'a (Mount and Baranski, 1953).

Diseases spread chiefly by arthropods

Resident physicians stated that *malaria* is one of the principal causes of sickness and death in the Yemen. Toffolon (1946) reported that *falciparum* malaria predominates over the other two common types throughout the year in the middle heights and in the Tihama, while *vivax* malaria is more commonly seen in the highlands. He found that the *Plasmodium* index of population groups in Ta'izz was lowest in May and June and began to rise rapidly in July and August, reaching the peak of the curve in November and December.

In the present survey, 289 thick and thin films have been examined as follows: 120 from Ta'izz; 100 from Hodeida; and 69 from San'a. Of these, 34 from Ta'izz, two from Hodeida, and one from San'a were positive. All three species were represented in the Ta'izz slides, *P. falciparum* and *P. malariae* in the Hodeida slides, and the positive slide from San'a was *P. vivax*. Parasite densities were usually low. The splenic indices of school children in the three groups parallel the *Plasmodium* indices quite closely: Ta'izz, 68.2 (45/66); Hodeida, 13.5 (17/126); and San'a, 2.1 (1/49). *Blackwater fever* was said to occur infrequently (Merucci, 1951).

The only anopheline infectivity studies thus far made in the Yemen were done in Ta'izz by Toffolon (1946) in 1944 and 1945. This study showed that *Anopheles gambiae* was naturally infected and on this and epidemiological grounds Toffolon considered it the principal malaria vector in Ta'izz. The results of Toffolon's epidemiological study in Ta'izz point to the open basins in the mosques used for ritualistic ablutions as the principal breeding sites for *A. gambiae*.

On general epidemiological evidence, Merucci (1951) is of the opinion that *A. adenensis* is the vector at Hodeida and *A. sergentii* is the vector at San'a. Madinat al Abid, a town on the Hodeida-San'a road at an elevation of about 4000 feet, is a notorious endemic malarious area. Here, Merucci found *A. gambiae*.

Plague has recently been reported from the northern Yemen near the Asir border (Amin, 1952). Both *Xenopsylla cheopis* and rats are common in urban communities throughout the country (Sanborn and Hoogstraal, 1952).

Sandfly fever is thought to occur in the Ta'izz district by the resident physi-

cians. While there, a number of sandflies were collected outside of the quarters occupied by the NAMRU-3 medical mission, and three members came down with an illness of short duration clinically resembling sandfly fever.

Relapsing fever is said to occur throughout both the middle heights and the highlands (Toffolon, 1951). The vector has not been identified. In 1929 an epidemic of relapsing fever occurred in San'a (Ganora, 1930), and in 1943 and 1944 epidemics of this disease occurred among soldiers and civilians in Ta'izz (Toffolon, 1946). Toffolon found 3.0 per cent of 420 persons with *Borrelia recurrentis* in their blood in 1943, while in 1944 examination of over 1500 persons showed that this organism was present in the blood of about 0.6 per cent of them.

While both the body louse, *Pediculus humanus humanus*, and the eyed tampan tick, *Ornithodorus savignyi*, are widespread in the Yemen (Hoogstraal), Merucci (1951) and Toffolon (1951) stated that they had never found *O. moubata*, a recognized tick vector of relapsing fever. Hoogstraal has not found this species in his Yemen tick collection.

Typhus fever has been reported to be prevalent in the San'a district (Petrie, 1939). Serological evidence indicates that both epidemic and murine typhus occur commonly in the middle heights and in the highlands (Mount and Baranski, 1953).

Nutritional diseases

Clinical avitaminoses are in evidence everywhere in the Yemen (Petrie, 1939), despite the abundance and variety of fresh vegetables and fruits throughout the year. Among the nutritional diseases reported by Petrie are *rickets*, *night-blindness*, *scurvy*, *pellagra* and *osteomalacia*.

Miscellaneous conditions

Vesical calculus formation is very common in all age groups (Merucci, 1951; Toffolon, 1951). Petrie (1939) reported the removal of calculi from the bladders of infants between one and two years of age. Nearly all cases occurred in the male sex.

No record of the occurrence of the following diseases in the Yemen has been found, nor was evidence of the presence of these diseases obtained during the present survey: *anthrax*, *cholera*, *dengue*, *leptospirosis*, *rabies*, *strongyloidiasis*, *trichinosis*, *yaws* or *yellow fever*.

Chewing of qat. On the basis of medical histories and observations, it is probable that about 80% of the adult male population of the Yemen chew qat leaves. Habitual users of qat stated that the active principle of the leaf acts as a mental and physical stimulant. Frequent complaints from adults giving a history of qat chewing of several years duration were loss of appetite, constipation, epigastric pain and sexual weakness.

DISCUSSION

This study of the health problems and associated conditions affecting the general welfare of the people in a country heretofore rather completely isolated,

while incomplete and inadequate for quantitative evaluations, provides a general picture of the health status of the people. It gives some idea of the regional distribution of the principal diseases, and of the agents and conditions which contribute to such diseases. However, since this mission was the first of its kind to operate in the Yemen and since its activities were carried on for a period of less than seven weeks, it is obvious that only the first step of disease elucidation in the Yemen has been accomplished.

The unusual relationship existing between the spread of malaria and schistosomiasis and the religious practices of the people (ritualistic ablutions) suggests that both of these serious health hazards would be very susceptible to control measures in urban communities.

The high incidences of intestinal parasitism and the intestinal group of bacillary diseases are probably in part due to fly dissemination. However, the water in the basins in the mosques could be an important source for general dissemination of the dysenteries and typhoid and paratyphoid fevers since the rinsing of the mouth, genitalia and anal area is a practice associated with the ritualistic ablutions. Furthermore, since the water supply throughout the Yemen is obtained from open wells or led from mountain streams to contaminated collection points and is stored in open receptacles in the homes, abundant opportunities for transmission of diseases such as typhoid and bacillary dysentery by means of public water supplies are present.

SUMMARY

A geomedical survey of three geographic regions of the Yemen was made during January and February, 1951. On the basis of this survey it would appear that there are at least 10 major disease problems in the Yemen, irregularly distributed through the principal geographic regions. In the lowlands (Tihama) as well as elsewhere, dysenteries, intestinal parasitism, venereal diseases, nutritional diseases, tuberculosis and eye diseases are common. This region has more malaria than the highlands but considerably less than the middle heights. Schistosomiasis occurs here to about the same extent as it does in the highlands but far less frequently than in the vicinity of Ta'izz in the middle heights. Typhoid fever appears to be fairly common in all three regions. In the middle heights, major disease emphasis is on schistosomiasis, malaria, veneral diseases, the dysenteries, tuberculosis, typhoid fever, typhus and trachoma. The highlands differ in that there is less schistosomiasis and malaria, and perhaps less dysentery.

REFERENCES

Amin, H. 1952, (Representative WHO, Regional Office for Middle East). Personal communication.

Bequaert, J., Mollusks from the Yemen. In preparation.

Buxton, P. A. 1944, Rough Notes: *Anopheles* Mosquitoes and Malaria in Arabia, *Trans. Roy. Soc. Trop. Med. Hyg.* **38:** 205–214.

De Meillon, B. 1947, The Anophelines of the Ethiopian Geographical Region, *Publ. S. Afr. Inst. Med. Res.* **10:** 1–272.

Ganora, R. 1930, Notizie sulla climatologia e nesografia dello Jemen (Arabia Felice) e su di un'epidemia di febbre ricorrente, *Arch. Ital. Sc. Med. Colon.* **11:** 475–479.

Hoogstraal, H., Ticks of the Yemen. In preparation.

Knight, K. L., Mosquitoes of the Yemen. In preparation.

Kuntz, R. E. 1952, *Schistosoma mansoni* and *S. haematobium* in the Yemen, Southwest Arabia: With a Report of an Unusual Factor in the Epidemiology of Schistosomiasis mansoni, *J. Parasit.* **38:** 24–28.

Kuntz, R. E., Malakatis, G. M., Lawless, D. K., and Strome, C. P. A. 1953, Medical Mission to the Yemen, Southwest Arabia 1951. II. A Cursory Survey of the Intestinal Protozoa and Helminth Parasites in the People of the Yemen, *Am. J. Trop. Med. and Hyg.* **2:** 13–19.

Merucci, L. 1951, Personal communication.

Mount, R. A. and Baranski, J. R. 1953, Medical Mission to the Yemen, SW Arabia, 1951. III. A bacteriological and serological survey. In preparation. *Am. J. Trop. Med. and Hyg.* **2:** 20–29.

Petrie, P. W. R. 1939, Some experiences in South Arabia, *J. Trop. Med. and Hyg.* **42:** 357–360.

Radford, C. D. 1952, Mites of the Yemen collected by the mission of U. S. Naval Medical Research Unit No. 3, 1951. In press. Fieldiana: Zoology.

Sanborn, C. C., and Hoogstraal, H. 1952, Mammals and their ectoparasites from the Yemen. In Press. Fieldiana: Zoology.

Sarnelli, T. 1935, La bilharziosi vesicale nello Yemen e nelle nostre colonie, *Arch. Ital. Sc. Med. Colon.* **16:** 410–424.

Toffolon, C. 1946, Malaria in Ta'izz. Unpublished paper.

Toffolon, C. 1951, Personal communication.

Traub, R., Fleas from the Yemen. In preparation for Fieldiana: Zoology.

MEDICAL MISSION TO THE YEMEN, SOUTHWEST ARABIA, 1951

II. A Cursory Survey of the Intestinal Protozoa and Helminth Parasites in the People of the Yemen[1]

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Since Arabia, until the past few years, has been more or less closed to westerners, it has been visited by only limited numbers of travelers or small expeditions with interests in exploration or archeology. More recently there has been concern with the development of industries and natural resources. The Yemen, in southwest Arabia, was even less frequently visited. There is very limited information relating to the diseases or health problems found in the Yemen because only a few persons with medical interests have penetrated into that part of the peninsula. However, following the treaties between the Iman Yehia and the Italian government in 1937, government agents and Italian medical officers found their way into the principal cities of the country. Unfortunately these doctors have made only a few documented contributions to the medical literature, the majority appearing in rather obscure Italian journals. The brief report by Petrie (1939), a British medical missionary working at a hospital in San'a by an arrangement between the King of the Yemen and the Aden government, seems to be the only account of general conditions pertaining to medicine. Sarnelli (1935) however did report on the finding of schistosomiasis in the Yemen.

Recently the King of the Yemen invited a group of investigators, the Naval Medical Mission to the Yemen from the U. S. Naval Medical Research Unit No. 3 at Cairo, to make a preliminary medical survey of the Kingdom. The group spent approximately seven weeks in the field during January and February, 1951, making studies and collections of material at three major, representative locations. These included: Hodeida, a Red Sea coast city in the Tihama or coastal plain with a population of 40,000; Ta'izz, the provisional capital situated in the intermediate zone at an elevation of 4100 feet with a population estimated to be 3000–4000; and San'a and Ma'bar on the highland plateau at 7200 feet. Ma'bar is a walled village occupied by several thousands of the typical pastoral

[1] The opinions or assertions contained therein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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The authors are indebted to Dr. Robert A. Mount, Medical Officer in Charge of the Naval Medical Mission to the Yemen for cooperation given in medical aspects of this study, to Harry Hoogstraal and Dr. Kenneth L. Knight for assistance in collecting molluscs, to J. R. Baranski for technical assistance in collecting parasitological materials, and to Abdel Aziz Salah Effendi who served in the capacity of recorder and interpreter.

inhabitants of the highlands whereas San'a is the country's largest city with an estimated population of 40,000 to 60,000 persons.

A report with map showing the route travelled by the Mission and a discussion of the general sanitary and health conditions has been given by Mount (1953) in the previous paper.

METHODS AND MATERIALS

Persons on whom data were collected at each of the locations on the Yemen trip were interviewed prior to examination by the medical officer to insure proper identification and to obtain pertinent medical history. Each was asked to contribute a sample of blood and blood smears for blood parasite examination. They also submitted perianal swabs (NIH type) and fecal and urine specimens.

Urine was collected in standard bottles and allowed to set for several hours until opportunity permitted examination. The greater part of the sample was decanted and the remaining portion transferred to conical sedimentation glasses. After 15 to 45 minutes 1 ml. of the sediment was examined under a compound microscope. Fecal specimens were taken in standard cardboard sputum cups. Unusual precautions were taken to make certain that specimens submitted to the recorder were those of the individual undergoing examination. Pea-size samples of feces were selected from different parts of the stool to insure a more representative specimen. Half of the specimen was preserved in a 20 ml. capacity, screw top vial containing 7.5 per cent formalin plus 1.5 per cent glycerine and the remainder was placed in a preparation of PVA (polyvinyl alcohol) for later study in the laboratory at Cairo. Vials containing feces were stirred thoroughly and shaken at intervals during the ensuing 24 hours to insure maceration and proper fixation of the entire sample. The perianal swabs were examined as they were made available.

A preliminary examination of a number of the fecal specimens in Cairo a few weeks later revealed that the samples preserved in PVA were far less useful for diagnostic purposes than those stored in formalin and glycerine. Consequently all protozoan and helminth determinations were based upon a study of the latter. Identification of the protozoa was made in the Department of Protozoology, using a "MIF" solution recently developed at NAMRU-3 (Sapero *et al.*, 1951). Members of the Department of Helminthology examined all fecal samples for helminth ova. Three coverslip (22 x 40 mm.) preparations were examined from each vial after thorough agitation.

FINDING AND DISCUSSION

The pertinent findings of this survey, based upon a single urine and fecal specimen and one perianal swab from each individual, are given in Table 1. No attempt was made to segregate data into sex, age or occupational categories since the samples were small and there were considerable differences in the numbers of patients examined at the four locations. As might be expected in an Arab country the majority of persons examined were males. The few women and girls appearing at the clinic came because they were ill and hoped to get imme-

diate relief for their ailments. Even these gave blood, urine and fecal specimens reluctantly. In countries such as the Yemen it is difficult to assign persons to a specific type of occupation.

Data collected are not based entirely on a random sampling of the population. For the sake of convenience and to conserve time, the Yemeni officials requested a given number of school children, or bandoleered infantrymen, to appear for examination. School children 7 to 16 years of age made up approximately 70

TABLE 1

Percentage of people infected with intestinal protozoa and helminth parasites (parasites identified from urine and fecal specimens, and perianal swabs)

PARASITES	LOCALITY			
	Hodeida	Ta'izz	Ma'bar	San'a
Intestinal protozoa				
Number of fecal specimens examined	72	100	26	24
Endamoeba histolytica	37*	33	65	37
Endamoeba coli	54	49	65	63
Endolimax nana	31	24	35	33
Iodamoeba bütschlii	19	31	42	75
Chilomastix mesnili	8	10	15	8
Giardia lamblia	19	11	0	13
Trichomonas hominis	4	2	0	0
Helminths				
No. fecal and urine specimens and perianal swabs examined	182	218	26	70
Ascaris lumbricoides	8	49	89	14
Enterobius vermicularis	7	14	21	26
Hymenolepis nana	18	4	4	10
Schistosoma haematobium	2	10	0	0
Schistosoma mansoni	7	56	4	18
Taenia saginata	0	0	16†	0
Trichuris trichiura	15	5	15	7

* Percentage infection is given as the nearest whole number.

† Two fecal specimens contained gravid proglottids as well as ova.

per cent of the overall sample taken and infantrymen ranging in age from 20 to 35 years, 10 to 12 per cent. The remainder consisted of officials and their families, and middle and old aged persons who came to the clinic voluntarily.

Our figures (Table 1) for percentages of infection with certain of the intestinal protozoa and helminth parasites are low if one takes into consideration the general sanitary conditions and the closeness of group and family associations characteristic of Arabic countries. However, it is a well recognized fact, as indicated by the statistical analyses of Svensson (1935) working with the identifica-

tion of protozoan parasites, that at least six and probably more stools are necessary to reveal an accurate percentage of infections in a population. In our survey the use of preserved fecal specimens probably introduces additional limitations for identification and rates of infection for protozoa.

The relationship between protozoan infections and the major representative localities from which materials were taken in the Yemen indicate an overall negligence and disregard for sanitation. Indiscriminate defecation by the majority of people and the nonexistence of sewage and garbage disposal systems fosters the maintenance and spread of enteric and parasitic diseases. It will be noted that the recorded rates of infection with protozoa are greater at Ma'bar and San'a located in the highlands than at Ta'izz and Hodeida. It is believed that the percentages of infection which are greater at Ma'bar than at other localities are an expression of the less hygienic habits and the greater crowding of these people rather than an indication of a differential due to geographic relationships. Similar rates of infection of *Endamoeba histolytica* at Hodeida, Ta'izz and San'a are a bit surprising since Hodeida is a seaport city with geographical and climatic features quite different from those inland. Furthermore it should be noted that during our visit we observed that the peoples of Hodeida were subjected to much greater densities of flies than were the populations at higher elevations. Although based upon specimens from a small number of people, the 75 per cent infection with *Iodamoeba bütschlii* at San'a is unusual.

In contrast to the intestinal protozoa, the incidence of helminth parasites in general seems to bear certain relationships to locality and elevation. The infections with *Ascaris lumbricoides* and *Taenia saginata* are examples. The rather low incidence of *Ascaris* (8 per cent) at Hodeida may be due in part to the much higher temperature recorded for this unprotected coastal city on the Red Sea. Such extreme temperatures, direct exposures to the sun and location of the city on a sandy plain surely are detrimental to the survival of *Ascaris* ova. High infections with *Ascaris* and *Trichuris trichiura* at Ma'bar, as with the protozoan infections, is probably due to a lack of personal sanitation and to the intimate contact among members of large families. In this respect however a higher percentage of infection would be expected for *Enterobius vermicularis* and *Hymenolepis nana*. The finding of *Taenia saginata* at Ma'bar only, certainly reflects the more common use of beef in this highland village. Pasture lands on the plateau encourage the raising of greater numbers of cattle whereas the limited vegetation, especially in that part of the Tihama in which Hodeida is situated, accounts for an absence of beef. Although people of Hodeida eat considerable quantities of marine fish and the shells of *Pirenella* (first intermediate host of *Heterophyes*) were found, ova from this or other trematodes transmitted by fish were not recorded. Opisthorchiid ova were found in the stool of one individual from San'a, and ova of *Heterodera* were observed in several fecal specimens from Ta'izz and Hodeida.

The infection by schistosomes presents the most interesting and important helminthological problem in the Yemen. There are two factors which may account for the presence and incidence of *Schistosoma mansoni* and *S. haematobium*. It seems that these parasites may have been introduced by the continual

migration of infected persons from Eritrea and other countries of East Africa to the Yemen, and that the snail vectors may, in some time past, have been carried into the country via the goat skin water bag which is always carried by Arabic nomads. The latter situation, at least, seems to be a factor in the current spread of snails in the Yemen. Religious practices, i.e., the washing of parts of the body in ablution pools in mosques prior to prayer is a second factor. Significance of the latter has been described recently by Kuntz (1952). Observations made on the Yemen trip indicate that the use of water in the open-top

FIG. 1. Ablution pools in mosque at Ta'izz. These serve as a source for infection with *Schistosoma mansoni* and water-borne enteric diseases.

ablution pools (Fig. 1) is the principal source of infection with *S. mansoni*. A similar potential hazard for infection by *S. haematobium* exists in the vicinity of San'a, where *Bulinus contortus* were found in open masonry cisterns so characteristic of the upland plateau, as well as in an ablution pool in the courtyard of a mosque. Furthermore it seems likely that the use of these grossly contaminated waters for washing teeth, gargling, etc. likewise contributes to infections with water-borne enteric diseases.

The snail vector of *S. haematobium* was not found at Ta'izz although 10 per

cent of the persons examined were positive for this infection. Unfortunately the rather crowded schedule of work at Ta'izz did not permit a survey of small streams 12 to 15 miles from the city where a number of the school children frequently swam and played. Three of the four *S. haematobium* infections at Hodeida were in men who had just recently come from Eritrea. *Biomphalaria boissyi arabica* were taken from several small streams 6 to 8 miles from Ta'izz but none were infected. *Biomphalaria*, containing developmental stages of schistosomes or passing cercariae, were found only in the mosques of Ta'izz (Kuntz, 1952). The majority of persons (7 per cent) from Hodeida with the ova of *S. mansoni* in their stools had come from Ta'izz or that general vicinity during the 18 months prior to examination. Similarly the single infection at Ma'bar was attributed to a person whose wanderings had taken him to the intermediate elevations toward Ta'izz. The same held true for the majority of persons found infected with *S. mansoni* at San'a. Our survey indicated that there were no schistosome vectors in the vicinity of Hodeida. Whether the infection is present on the upland plateau at San'a is questionable since no *Biomphalaria* and *Bulinus*, even in locations favorable for infection, were found to have schistosome parasites.

It should be noted that the first *S. haematobium* infection was revealed from a perianal swab. After all data were recorded it was learned that approximately 10 per cent of all *S. mansoni* and six of 24 *S. haematobium* infections were detected by ova on the perianal swabs. This, however, is an expected finding in making a survey in Arabic countries where there is little or no use of toilet paper among the native populations.

An engorged leech, *Limnatis paluda*[1] (Tennent, 1859), was removed from the naso-pharynx of one man at Ta'izz who came to the clinic complaining of an unusual irritation in his throat. This species was found in several water storage basins and in springs used by man and herds of goats and sheep.

No evidence of hookworm infection was detected in the Yemen. This seems worthy of note since some texts on parasitology and tropical medicine, e.g. Manson-Bahr (1950) imply that the hookworm has been found so widely distributed that it may be assumed to occur in all tropical and subtropical countries. Seemingly favorable conditions of temperature and humidity at Ta'izz and similar locations in the Yemen in conjunction with the migration of considerable numbers of persons from parts of East Africa, and especially India, certainly would lead one to suspect the presence of hookworm. An Italian doctor (Merucci, 1951) informed us that he had observed hookworm infections in only two persons during his 14 years of residence in the Yemen. He likewise had looked for hookworms and expressed surprise at the paucity of infections in the Yemen.

Although *Wuchereria bancrofti* and *Dracunculus medinensis* are presumably quite common in Arabia, these parasites were not encountered on this brief visit and survey of the Yemen. The use of wells with steps leading down and into the water and the use of water from the open cisterns so common in the Yemen cer-

[1] Leeches collected in the Yemen have been studied and identified by Dr. Marvin C. Meyer, Department of Zoology, University of Maine, Orono, Maine.

tainly provide acceptable requisites for propagation of the guinea worm's cycle. It must be stated however that there is evidence of drying up and lowering of the water table in southwest Arabia which has resulted in the use of fewer wells suitable as sources for *Dracunculus* infection.

SUMMARY

The first parasitological survey of the Yemen, Southwest Arabia has made known the presence of certain parasites of man in a part of the world which until recently has been seldom visited by westerners. Our findings extend the known boundaries of distribution for *Schistosoma mansoni* and *S. haematobium* and reveal an unusual factor in the epidemiology of schistosomiasis mansoni. Rather high rates of infection with *S. mansoni* in the Yemen are attributed to the use for religious purposes of infected waters in ablution pools of mosques. *Ascaris*, *Enterobius*, *Trichuris* and *Hymenolepis* are quite common and *Taenia saginata* was found in the people of Ma'bar on the San'a plateau. Infections with hookworm, *Wuchereria* and *Dracunculus* were not recorded during this brief visit to the Yemen.

REFERENCES

Kuntz, R. E. 1952, *Schistosoma mansoni* and *S. haematobium* in the Yemen Southwest Arabia with a report of an unusual factor in the epidemiology of schistosomiasis mansoni, *J. Parasit.* **38:** 24–28.

Manson-Bahr, P. H. 1950, Manson's Tropical Diseases. Cassell and Company, Ltd.

Mount, R. A. Medical mission to the Yemen, Southwest Arabia, 1951. I. Geomedical observations, *Am. J. Trop. Med. and Hyg.* **2:** 1–12.

Merucci, L. 1951, Personal communication.

Petrie, P. W. R. 1939, Some experiences in South Arabia, *Trop. Med. and Hyg.* **42:** 357–360.

Sapero, J. J., Lawless, D. K. and Strome, C. P. A. 1951, An improved iodine staining technique for routine laboratory diagnosis of intestinal protozoa, *Science* **114:** 550.

Sarnelli, T. 1935, La bilharziosi vesicale nello Yemen e nelle nostre colonie, *Arch. Ital. Sc. Med. Colon.* **16:** 410–424.

Svensson, R. 1935, Studies on human intestinal protozoa especially with regard to their demonstrability and the connexion between their distribution and hygienic conditions, *Acta Med. Scand.* suppl. **70:** 1–115.

MEDICAL MISSION TO THE YEMEN, SOUTHWEST ARABIA, 1951

III. A Serological and Bacteriological Survey[1]

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The present study formed a part of a geomedical survey made in the Yemen during January and February of 1951 (Mount, 1953), and is the first bacteriological and serological survey to be reported from this section of the Arabian Peninsula.

With few exceptions, published data pertaining to the prevalence and distribution of diseases in the Yemen are based on clinical observations because laboratory diagnostic facilities are not generally available. Since clinical cases frequently represent only a small percentage of the total infections, serological and cultural studies carried out on well population groups should give a more accurate picture of the distribution and prevalence of many diseases in which subclinical infections occur.

Bacteriological cultures were made on the majority of stool specimens submitted at the three representative geographic localities visited, and blood samples were taken in Ta'izz and San'a.

MATERIALS AND METHODS

Population sampling

Random sampling of the population groups residing in the localities visited was not attempted. Women and children under school age could not be included in the series (Kuntz *et al.*, 1953). Since neither zone maps of urban areas nor census figures were available, unbiased sampling of older age groups could not be done in the time at our disposal. The best feasible means of getting well persons in the younger age groups for the survey series appeared to be by the inclusion of school children and soldiers. The remainder of individuals in the series were selected from government officials and members of their families who came to the clinic for treatment. Those obviously ill were excluded from the population samples. Each individual was interviewed and a brief medical history was taken prior to a physical examination and the collection of specimens.

Field collections and processing

Blood samples were taken by means of 10 ml. capacity vacuum collection tubes and allowed to clot at room temperature. The clot was freed and spun down at 1200 r.p.m. for 12 to 15 minutes, the serum withdrawn and stored in pyrex

[1] The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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tubes at −10° to −20°C. until the return of the mission to NAMRU-3 in Cairo, Egypt.

Fecal specimens were streaked on *Shigella-Salmonella* agar (SS, Difco) plates, which were incubated at 37°C. for 18–24 hours. Typical non-lactose-fermenting colonies were transferred to triple sugar-iron agar (TSI, Difco) slants, incubated as before, and beef heart infusion agar (BHI, Difco) butts were inoculated from the slants. These were sealed with paraffinized cork stoppers, packed in boxes and stored at room temperature for subsequent study in the Bacteriology Department of NAMRU-3 in Cairo.

Studies in Cairo

Serology. *Brucella melitensis* agglutination tests were performed on 260 sera: 193 collected in Ta'izz and 67 in San'a. Using the macroscopic test, twofold dilutions of serum from 1:20 through 1:320 were titrated against a standardized suspension of *B. melitensis* prepared by the method advocated by Wadsworth (1947). The endpoints were taken as the highest dilution showing three or four-plus agglutination. *Salmonella typhi* O and *Salmonella* non-specific H (NSH) agglutination reactions (Todd and Sanford, 1943) were done on 218 sera: 161 from Ta'izz and 57 from San'a[4]. Standard Kahn flocculation tests (Todd and Sanford, 1943) were done on 260 sera. These were the same serum samples that were tested against *Brucella* antigen. Sera showing a two plus or greater flocculation (average of six readings, making two readings 15 minutes apart of the three tubes used in the test) were recorded as positive. Since there was no appreciable difference in the per cent of Kahn positive sera obtained from Ta'izz and San'a, the results were compiled collectively. *Proteus* OX-19 and *Proteus* OX-K agglutination tests were performed on 198 sera from Ta'izz and 67 serum samples taken in San'a. The test tube reaction, using heat-killed phenolized antigen suspensions (Amer. Pub. Health Assoc., 1945), was employed in both titrations. Complement fixation tests with washed (specific) epidemic and murine typhus antigens[5] (Amer. Pub. Health Assoc., 1948) were performed on 113 of the above sera. These were selected without reference to the results of the *Proteus* agglutination reactions, 69 from the Ta'izz samples and 44 from those collected in San'a.

Bacteriological studies. Upon return to Cairo, the stock BHI cultures were streaked on both SS and MacConkey agar plates and incubated at 37.0°C. for 18 to 24 hours. Typical colonies were picked from the plates and streaked on BHI slants. Following a similar period of incubation, growth was inoculated into the usual sugars for biochemical differentiation. All cultures which corresponded biochemically with the *Shigella* group of organisms were typed by the use of specific sera[6]. Duplicate stock cultures were sent to the bacteriology facilities

[4] The antigens used were purchased from the Central Public Health Laboratory of the Public Health Laboratory Service in London, England.

[5] Furnished by the Division of Virus and Rickettsial Diseases, Army Medical School, Washington, D. C.

[6] Purchased from the Central Public Health Laboratory, Public Health Laboratory Service, London, England.

of the U. S. Navy Medical Research Institute (NMRI) for confirmatory typing, along with stock cultures of organisms which belonged biochemically to the *Salmonella* group.

FINDINGS AND DISCUSSION

No attempt was made to divide the data into categories other than age and location for reasons already mentioned (Kuntz *et al.*, 1953). It had been planned originally to carry out a more comprehensive serological study than was done. Our inability to do so was due mainly to defective collection tubes. This factor so reduced the quantity of serum obtained from the majority of persons that neutralization tests in mice and additional complement-fixation reactions had to be deleted from the protocol. Complete *Salmonella* agglutination studies could not be done.

The *Brucella* agglutination reactions (Table 1) suggest that brucellosis is of more common occurrence in the Yemen than clinical observations by resident physicians (Toffolon, 1951) would indicate. Lack of recognition of this disease

TABLE 1

*Brucella agglutination reactions on the sera of 260 individuals at Ta'izz and San'a in the Yemen**

	Per cent of sera agglutinating at, or above†				
ANTIGEN	1:20	1:40	1:80	1:160	1:320
Brucella melitensis	3	3	2	1	1

* 193 sera collected at Ta'izz.
67 collected at San'a.

† Percentages are given as the nearest whole number.

is not surprising in a country where diagnostic laboratory facilities are lacking, and where malaria, relapsing fever, typhoid and tuberculosis are reported to be prevalent (Petrie, 1939). Since two per cent of the sera had agglutination titers of 1:80 or higher against *B. melitensis*, the implication is that at the time of examination some of the positive reactors either had brucellosis or had recently recovered from an acute attack (Harris, 1950). However, since none were obviously ill, the higher titers may have been the result of frequent subclinical infections. Wilson and Miles (1946) point out that this latter situation may exist in endemic areas and usually occurs in persons drinking large quantities of raw milk or exposed to contact with infected animals or carcasses. In this connection, it is noteworthy that goat raising for hide exportation is a major industry at middle elevations and in the highlands of the Yemen.

The results of *Salmonella* NSH and *S. typhi* O agglutination tests (Table 2 and Graph 1) indicate that *Salmonella* infections are frequent in all age groups tested and suggest that initial attacks of typhoid and paratyphoid fevers occur early in life, the agglutinin titers being maintained subsequently by repeated infections. The increase in the percentage of sera from persons in the 21 to 30

years age group with detectable NSH agglutinins in the face of a definite decrease in the percentage of *S. typhi* O positive in the same group is worthy of comment, particularly in view of the reversal which occurred in persons over 30 years of age. While it is possible that these apparent differences in the age group distribution of the two agglutinins are due to the more rapid loss of O as compared to H agglutinins following infection (Wilson and Miles, 1946), this explanation requires an additional factor which would lessen the exposure of the 21 to 30 years age group to infection in comparison to all other age groups included in the population sample. That the exposure of this group was less is suggested by the stool culture findings (Table 3). It may be observed that the lowest percentage of fecal samples found positive for enteric pathogens occurred in the samples submitted by the age group in question. As stated previously the majority of persons examined were school children and soldiers. With few excep-

TABLE 2

The percentage distribution of Salmonella *non-specific "H" agglutinins and* S. typhi *"O" agglutinins by age group and location in the Yemen*

	PER CENT OF SERA AGGLUTINATING AT 1:20 OR ABOVE, BY AGE GROUPS*				
Age in years	11–15	16–20	21–30	over 30	Total
No. sera from					
Ta'izz (number)	31	30	59	42	162 } 218
San'a "	14	31	4	7	56 }
Salmonella N.S.H (per cent)	13	13	22	8	15
Ta'izz "	13	12	24	10	16
San'a "	14	13	0	0	11
S. typhi "O" "	70	84	51	63	67
Ta'izz "	58	93	47	60	61
San'a "	93	76	100	86	82

* Percentages are given as the nearest whole number.

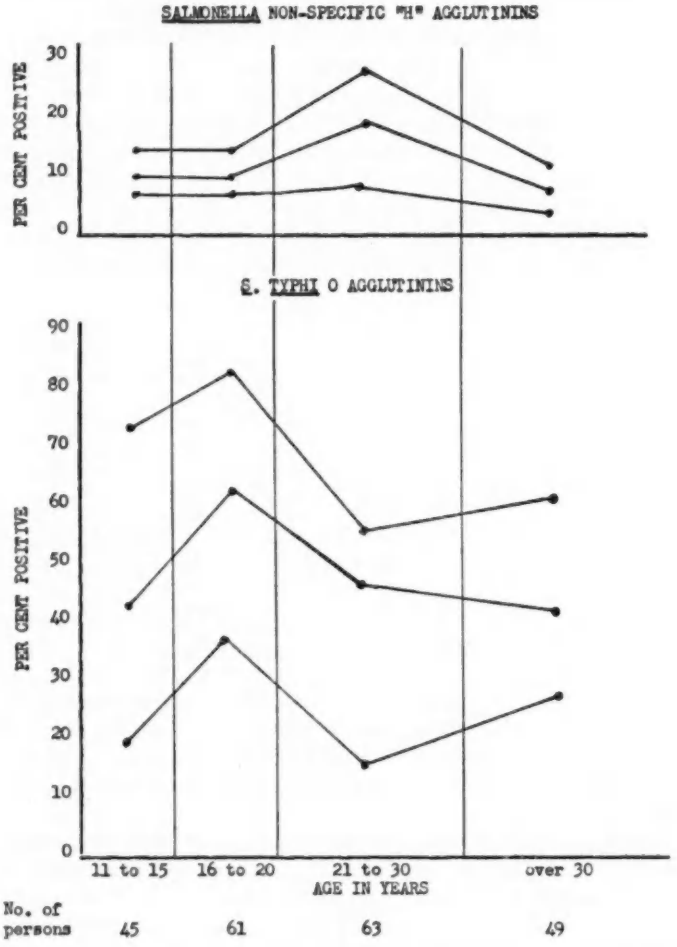
tions, soldiers made up the 21 to 30 years age group. While proof is lacking, one suspects that the occupational differences, coupled with the regimentation and discipline incidental to military life, would lessen the chances of persons in this group acquiring enteric infections even though modern sanitation is not generally practiced.

The large number of *Shigella* isolations obtained by single stool cultures (Table 3 and 4) indicates that bacillary dysentery is very prevalent in the Yemen. Since intermittency of excretion of organisms of this genus occurs in convalescents and carriers (Cruickshank and Swyer, 1940), the number of isolations made during the present survey is probably far lower than the figure obtainable by repeat cultures. The distribution and prevalence of the different species and types of *Shigella* and *Salmonella* show rather striking differences in the two groups of comparable size, namely, those from Hodeida and Ta'izz (Table 4). The greatest difference is in the number of *S. sonnei* isolations from the two localities.

In Ta'izz, these comprised one-half of the *Shigella* isolations; whereas only a single isolation of this organism was made from the cultures taken at Hodeida.

GRAPH 1

The distribution of Salmonella *nonspecific H agglutinins and* S. typhi *agglutinins in sera collected from 218 persons in Ta'izz and San'a*



In contrast, the Hodeida cultures yielded five salmonellas, four of these being *S. typhi*, as opposed to a single *Salmonella* isolation from the Ta'izz cultures.

The high percentage of persons with positive Kahns among those contribution blood samples in Ta'izz and San'a (Graph 2) indicates that treponematosis is

very prevalent in the Yemen. Petrie (1939) reports that syphilis is common and widespread, and that while bejel is present it is found principally in the hilly districts inland from Zabid. Both Toffolon (1951) and Merucci (1951) are of this opinion. While no recognized cases of bejel were seen by members of this mission, the even distribution of persons with positive Kahns in all age groups

TABLE 3

Distribution by age groups of Shigella *and* Salmonella *types in the Yemen on the basis of a single stool culture*

	AGE IN YEARS					TOTAL
	6–10	11–15	16–20	21–30	over 30	
No. examined	43	97	64	94	60	358
Per cent of pop. sample	12	27	18	26	17	100
Pos. for *Shigella*	6	12	12	10	11	51
Shigella						
ambigua					3	3
sonnei		2	5	5	6	18
flexneri						
I	2		1			3
II	1	2	2	1	1	7
III	1	1	2	1	1	6
IV						
V		4	1	1		6
VI	1	1		1		3
VII						
VIII				1		1
sachs	1	2				3
dispar			1			1
Salmonella	4	1	2	1		8
paratyphi						
A	1		1			2
B			1	1		2
C						
typhi	3	1				4
Total *Shigella* & *Salmonella*	10	13	14	11	11	59
Per cent pos. for enteric pathogens*	23	13	22	12	18	17

* Percentages are given as the nearest whole number.

(Graph 2) leads one to suspect that an appreciable percentage of the positives are due to this disease rather than to syphilis. In view of the marked differences in geography and climate between the isolated mountainous regions of the Yemen and the desert lands of Saudi Arabia and the Middle East where bejel is common among nomadic tribes (Napier, 1946), detailed studies of the disease

as it occurs in the Yemen might shed additional light on the relationship of bejel and syphilis. Petrie (1939) states that during the two years spent in San'a he did not encounter a single case with neurological manifestations.

The large proportion of sera tested with complement-fixing antibodies against both murine and epidemic typhus antigens (Table 5) and with positive Weil-

TABLE 4

Regional distribution of Shigella *and* Salmonella *types in the Yemen on the basis of a single stool culture*

	LOCATION			
	Hodeida	Ta'izz	San'a	Totals
No. examined	159	160	39	358
No. positive for:				
Shigella	14	34	3	51
ambigua	1	2	0	3
sonnei	1	17	0	18
flexneri				
I	2	1	0	3
II	1	5	1	7
III	2	2	2	6
IV	0	0	0	0
V	1	5	0	6
VI	2	1	0	3
VII	0	0	0	0
VIII	0	1	0	1
sachs	3	0	0	3
dispar	1	0	0	1
Salmonella	5	1	2	8
paratyphi				
A	1	0	1	2
B	0	1	1	2
C	0	0	0	0
typhi	4	0	0	4
Total *Shigella* & *Salmonella*	19	35	5	59
Per cent found positive*	12	22	13	

* Percentages are given as the nearest whole number.

Felix reactions (Table 6) shows that typhus is highly endemic in the mountainous regions of the Yemen where the sera were obtained. If these findings reflect the experience of the general population residing at the middle elevations and in the highlands, there is little likelihood of typhus ever occurring in epidemic proportions in these geographic locations, so long as conditions affecting transmission of this disease in the Yemen remain the same. With the exception of spotty

cowpox vaccinations over the country, no preventive inoculations are given, so that the demonstration of complement-fixing (CF) antibodies using washed

GRAPH 2

Percentage of Kahn positive sera collected from 260 individuals examined at Ta'izz and San'a

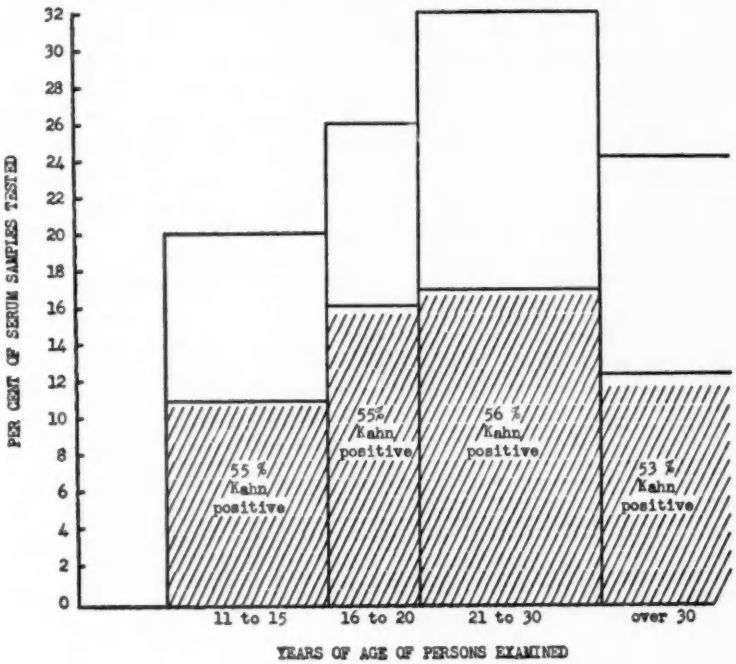


TABLE 5

Complement fixation tests on 113 sera collected at Ta'izz and San'a using epidemic and murine typhus antigens

ANTIGEN	SERUM DILUTIONS		
	Per cent sera positive at, or above*		
	1:8	1:16	1:32
Epidemic	22	17	8
Murine	28	24	21
Total per cent positive	50	41	29

* Percentages are given as the nearest whole number.

typhus antigens is taken as evidence of past infection. Since the agglutinin titer against the *Proteus* OX-19 strain falls rapidly in convalescence (Wertman, 1948),

the high per cent of the sera with agglutinin titers of 1:80 or above suggests that frequent infections occur. The preponderance of murine over epidemic CF positives in the highest dilution used (Table 5) is not surprising in view of the large domestic rodent population (Sanborn and Hoogstraal, 1952). It appears probable that flea transmission may play the major role in the epidemiology of typhus in the Yemen, in contrast to the situation usually encountered in typhus outbreaks among the desert Arabs; nomadic habits, meager fare and shortage of water (except for drinking) favor louse transmission (Mount and Salah, 1952).

The OX-K agglutination reaction findings (Table 6) are somewhat surprising. Although relapsing fever may result in the formation of OX-K antibodies (Zarafonetis *et al.*, 1946) and has been reported to occur both in San'a (Ganora, 1930; Petrie, 1930) and in Ta'izz (Toffolon, 1946), the last recognized outbreak of this disease in Ta'izz was in 1944 (Toffolon, 1951). It seems unlikely that recent widespread outbreaks in this locality could have escaped recognition. Furthermore, since the disease was occurring in both Ta'izz and San'a, it would be expected that if the OX-K reactions were due to past infection with *Borrelia recurrentis*,

TABLE 6

Weil-Felix tests on 265 sera collected at Ta'izz and San'a against Proteus *OX19 and OXK antigens*

ANTIGEN	SERUM DILUTIONS			
	Per cent sera positive at, or above*			
	1:40	1:80	1:160	1:320
OX19	32	17	6	1
OXK†	18	9	2	1

* Percentages are given as the nearest whole number.

† All positives wre among the sera collected at Ta'izz.

some of the San'a sera would have been OX-K positive. While mite typhus has not been reported from either the Arabian peninsula or the African continent, the possibility that some form of this disease is endemic in the Ta'izz district must be considered. The presence of several species of Trombiculid mites in the collections from wild rodents and small game trapped in the Yemen (Radford, 1952) is in keeping with this possibility.

SUMMARY

Bacteriological studies, carried out on 358 single fecal samples from persons residing in three geographically different locations in the Yemen, indicate that both *Salmonella* and *Shigella* infections occur commonly at all these locations. Serological studies, using *Salmonella* nonspecific H and *S. typhi* O antigens, suggest that both typhoid and paratyphoid fevers are highly endemic.

Brucella melitensis agglutination reactions on the serum of 260 persons in Ta'izz and San'a show that brucellosis is fairly prevalent in these localities, over 3 per cent having serum agglutinin titers of 1:40 or greater.

Standard Kahn tests were done on 260 sera collected at Ta'izz and San'a. Over 50 per cent of the sera were positive in all age groups included in the survey series.

Weil-Felix and complement fixation reactions on sera collected from persons residing in the mountainous districts of the Yemen indicate that both murine and epidemic forms of typhus are highly endemic in this section of the country. Positive OX-K agglutination reactions on the sera collected at Ta'izz raise the possibility that some form of mite typhus may be present.

REFERENCES

American Public Health Association 1945, Diagnostic Procedures and Reagents. Second Edition, pp. 146–151.

American Public Health Association 1948, Diagnostic Procedures for Virus and Rickettsial Diseases. First Edition, pp. 319–320.

Cruickshank, R. and Swyer, R. 1940, An outbreak of Sonne dysentery, *Lancet* Dec. 28, No. 6122, 803–805.

Harris, H. J. 1950, Brucellosis (Undulant Fever) Clinical and Subclinical. Second Edition, Paul B. Hoeber, Inc. New York., ch. VI, 276–294.

Kuntz, R. E., Malakatis, G. M., Lawless, D. K., and Strome, C. P. A., 1953. Medical Mission to the Yemen, Southwest Arabia, 1951. II. A Cursory Survey of the Intestinal Protozoa and Helminth Parasites in the People of the Yemen, *Am. J. Trop. Med. and Hyg.* **2:** 13–19.

Merucci, L., 1951. Personal communication.

Mount, R. A. 1953, Medical mission to the Yemen, Southwest Arabia, 1951. I. Geomedical observations, *Am. J. Trop. Med. and Hyg.* **2:** 1–12.

Mount, R. A., and Salah, A. A. 1952, A typhus outbreak in Cyrenaica, Lybia. Unpublished special report to W.H.O.

Napier, L. E. 1946, The Principles and Practice of Tropical Medicine. The Macmillan Company, New York, 539–540.

Petrie, P. W. R. 1939, Some experiences in South Arabia, *J. Trop. Med. and Hyg.* **42:** 357–360.

Radford, C. D. 1952, Mites of the Yemen collected by the mission of U. S. Naval Medical Research Unit No. 3, 1951. In press. Fieldiana: Zoology.

Sanborn, C. C., and Hoogstraal, H. 1952, Mammals and their ectoparasites from the Yemen. In press. Fieldiana: Zoology.

Todd, J. C., and Sanford, A. H. 1943, Clinical Diagnosis by Laboratory Methods. A Working Manual of Clinical Pathology. W. B. Saunders Company, Philadelphia. 659–662; 696–698.

Toffolon, C. 1946, Malaria in Ta'izz. Unpublished paper.

Toffolon, C. 1951, Personal communication.

Wadsworth, A. B. 1947, Standard Methods of the Division of Laboratories and Research of the N. Y. Depart. of Health. Third Edition. Williams and Wilkins, Baltimore.

Wertman, K. 1948, The Weil-Felix Reaction. Rickettsial Diseases of Man. A Symposium. American Assoc. for the Advancement of Science. Washington, D. C. Moulton, F. R., Editor, 184–189.

Wilson, G. S., and Miles, A. A. 1946, Topley and Wilson's P[illegible]ples of Bacteriology and Immunity. Third Edition. Edward Arnold and Co., Lond[illegible]ol. 11, 1536; 1704.

EPIDEMIOLOGICAL SURVEY AMONG THE GOLA TRIBE IN LIBERIA

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INTRODUCTION

Differences in customs, practices and ancestry have resulted in eighteen different tribal groups in Liberia, each with its own dialect. Some of the tribes living in adjacent areas show evidence of a common or closely related ancestry, as reflected in the sound and intonation of their basic spoken words and in some of their manner of play, worship and gestures of sorrow.

Northwest of the St. Paul River and northeast of Grand Cape Mount County is a vast area known as the Western Province. This Province is occupied by six large tribal groups without definite boundary. The tribes are Gbandie, Loma, Bele, Mende, Gola and Gizzie. This article deals primarily with the clinical findings from a series of surveys and studies made among the Gola tribe. The ethnic group known as the Golas makes up the dominant element of those living in an area of about 3,000 square miles, extending from the northwest bank of the St. Paul River, at about 30 miles from the Atlantic coast, to the Mano River, a distance of about 50 miles. In this area is the great Gola Forest with its fine timber and few people. In recent years tribal lines and many social customs have been altered by more frequent and friendly intercourse, better transportation and a spread of more uniform educational activities.

The Gola tribe is very interesting for study because only within the last two decades have large numbers of individuals had direct contact with the coastal cities where the ideas of western culture and practices are more evident. Facts are virtually nonexistent with regard to their origin. Expression of thought among the Gola tribe is done primarily by the spoken dialects with the liberal use of signs and symbols. Within the last few years, attempts have been made to preserve some of the cultural aspects of the Golas by developing the dialects into a written language. The alphabetic characters employed are still bizarre.

SURVEY

The survey outlined in this report is a study of some 3,000 persons of the Gola tribe, who at the time of the survey inhabited the area referred to as the Gola area of the great Gola Forest. This group consisted of individuals of all ages, sexes and political status who fell into the following age-sex group:

Age:	Children	1650 or 55%
	Adults	1350 or 45%
Sex:	Males	1840 or 61%
	Females	1160 or 39%

It was difficult to give a more exact age classification.

Malaria

Malaria is the most important infectious disease problem of the Gola tribe. From a microscopic study of a single thick blood smear taken from each of 2,500 Gola individuals of all ages and of both sexes, stained by the Wright-Giemsa method as described by Michelson and Wilcox (1940), 375 or 15 per cent were positive for plasmodia. As one would expect, the incidence of parasitemia varies greatly with age. From the smears taken from 1,350 adults there were 149 or 11 per cent positive for plasmodia, while similar smears taken from 1,150 children gave 224 or 19 per cent positive. An additional 6 per cent of the children showed clinical evidence of malaria even though a single thick smear was negative for plasmodia. The proportion of positive bloods in adults did not differ significantly according to sex, while in children there was a significant difference. Among 648 boys, 25 per cent of the blood smears were positive; but only 13 per cent were positive among 502 girls. In one church mission boarding school in the Gola district, the relative parasitemia discovered was about 5 times higher in 64 boys than in 100 girls of the same ages. A casual inspection showed three probable explanations: (1) the boys' sleeping quarters were not screened, (2) their quarters were on the side of the campus nearest the swamps in which no control of mosquito breeding was practicable, and (3) on rainy nights the boys had unrestricted permission to go snail hunting and crabbing without any protection from mosquitoes.

Ninety-one per cent of the positive smears showed *Plasmodium falciparum* trophozoites alone; 6 per cent showed *P. falciparum* trophozoites and gametocytes and 3 per cent showed *Plasmodium malariae* trophozoites or gametocytes. Neither *Plasmodium vivax* nor *P. ovale* was found in this study.

In the adult, the classic symptoms of chills, fever and sweats are rarely seen together. The patients more often complain of fever and headaches at night, painful joints and a bitter taste in the mouth upon arising. Acute attacks of malaria are rare and, as shown above, only 11 per cent of the adults gave positive blood smears. In the infants and in the young children the classic symptoms of malaria are the rule rather than the exception. They have chills, fever, sweats, gastro-intestinal disturbances and occasionally a lethargy approaching stupor. If not promptly treated, death frequently occurs.

Treponemiasis

Treponemiasis was diagnosed by clinical and laboratory methods. Blood was collected by Keidel tubes and the collection for one day was despatched to the laboratory in Monrovia by runner and canoe during the night when the heat was less. These blood samples reached the USPHMIL Laboratory the next morning where the serology tests were done. Kahn tests were made on 1,500 individuals. Two hundred ninety-nine or 20 per cent of these tests were strongly positive and represent cases of yaws or syphilis, since malaria and leprosy had been identified and excluded. All of the cases showed clinical signs compatible with a diagnosis of yaws, but a concurrent syphilitic infection in a small number cannot be ruled

out, since it appears that *Treponema pertenue* infection confers only a partial immunity against *Treponema pallida*. Twenty-three cases of clinical treponemiasis were observed among those who gave weakly positive or negative Kahn reactions. In these the skin, joint or bone involvement were quite characteristic of old cases of yaws whose serological reactions have been modified by inadequate anti-yaws medication. Thus 322 cases of treponemiasis were found, or 21 per cent of the cases studied. The great majority of these cases were yaws, with the following three types predominating:

Crab yaws—in which hyperkeratosis involving the tissues of the soles of the feet was the chief superficial lesion.

Dry yaws—in which the rubber-like dry skin of the forearm, hands and ankles with changes in pigmentation was the chief superficial lesion.

Acute frambesia—with many mulberry-like painful lesions on several parts of the body. These lesions are more common among children with the majority of the lesions on the face, buttocks and thighs.

Cases in which the chief pathology appears to be in the bone, joint and mucous membranes were rarely seen.

A selected group of 100 adolescent boys living under unusually unsanitary conditions in this tribe showed 34 per cent positive Kahn tests, while 100 adolescent school girls living in dormitories of a church mission in the same area showed 2 per cent positive. This is a significant difference. The chief factors here are less contact exposure to infected lesions and better bodily hygiene among the girls.

Intestinal helminthiases

A single fresh unconcentrated direct fecal smear examination was made on stools from 950 individuals. The method used was similar to that described on page 683 of "Laboratory Methods of the U. S. Army" by Simmons and Gentzkow (1948), except that to one drop of the comminuted feces on the slide a drop of D'Antoni's iodine stain was added for microscopic study. These examinations were done in the field using stumps, logs or boxes upon which to rest the microscope.

Intestinal helminth ova or larvae were present in 589 or 62 per cent of the stools examined. The incidence varied with the age of the patient. Fecal smears from 610 children were positive for helminth ova or larvae in 518 cases, or 85 per cent, while those from 340 adults were positive in only 71 cases, or 21 per cent. The highest incidences were found in the 100 boys mentioned above, of whom 98 per cent were positive, but of the 100 girls in the comparison group, only 54 per cent were positive.

The relative prevalence of the 5 dominant intestinal helminths in a group of 100 girls and 100 boys was as follows:

	Boys	*Girls*
Ascaris lumbricoides ova	58%	66%
Hookworm ova	53%	36%
Strongyloides stercoralis larvae	3%	10%
Trichocephalus trichiura ova	2%	4%
Enterobius vermicularis	1%	2%

Sixteen per cent of the boys had multiple intestinal helminth infections. The field methods used in these stool examinations were not sufficiently accurate to justify including the findings on the incidence of intestinal protozoa.

Dermatitis and dermatoses

Among the 3,000 persons studied there were 960 which showed one or more types of skin pathology. There was a wide variety of dermatological lesions. These included several types of mycotic infections, tropical ulcers and pyodermas with inconstant microbic flora, scabies with and without secondary pyodermas, hyperkeratotic and frambesiform lesions of yaws, skin lesions of leprosy and a miscellaneous group of skin lesions including keloids, varicose veins with ulcerations, several types of tribal skin markings, skin reaction to insect bites and a few cases of contact and heat dermatitis. These dermatological conditions were present in 960 or 32 per cent of the 3,000 persons studied. Table 1 gives the distribution of these cases.

TABLE 1

Etiological or clinical distribution of 960 patients with one or more skin lesions

TYPE OF LESION	NUMBER OF CASES	PERCENTAGE OF TOTAL*
Mycotic	461	48
Tropical ulcer and pyodermas without scabies	298	31
Scabies	57	6
Yaws	21	2
Leprotic	5	0.5
Miscellaneous	221	23

* Several patients had more than one type of skin lesion.

All of the laboratory identifications in this section were done at the USPHMIL Laboratory in Monrovia. Skin scrapings from all suspected dry mycotic lesions were collected in properly cleaned small ointment boxes. These scrapings were plated and if fungi were present they were isolated and identified. This identification was often done many weeks after the field collection. Several keys and reference books were used in the identification with the following three in most frequent use: Dodge (1935) Conant *et al.* 1944, and Ash and Spitz (1947). All specimens were submitted to three types of laboratory study: aerobic and anaerobic cultivation on Sabouraud's medium and corn meal agar, and hanging drop studies using 25 per cent potassium hydroxide.

From the moist lesions suspected of being mycotic, the inoculation was made on sterile corn meal agar tubes in the field and the study completed later as described above. For other dermatological lesions requiring laboratory confirmation such as leprosy as well as some of the ulcers and pyodermas, the smears were taken and stained in the field and studied in detail later.

A more detailed classification of the etiological agents of the 461 cases of mycotic infection is given in Table 2.

In addition to the 298 cases of tropical ulcers (Poindexter, 1950) and pyoder-

mias without scabies mentioned in Table 1, there were 83 more cases of scabies, yaws or leprosy which exhibited pyodermatoses. This made a total of 381 cases; their distribution and dominant microbic flora are shown in Table 3.

TABLE 2

Clinical and etiological classification of 461 cases with mycotic lesion of the skin

NAME OF DISEASE	ETIOLOGICAL AGENT	NO. OF CASES	PERCENTAGE OF TOTAL
Pityriasis versicolor	*Malassezia furfur*	318	69
Ringworm, scalp and bearded regions	*Trychophyton Schoenleinii*, *Microsporum lanosum*, *Trichophyton violaceum* and other *Trichophyton* spp.	69	15
Trichophytosis of feet, hands and body	*Trichophyton gypseum*, *Trichophyton purpureum*, *Trichophyton concentricum*; *Trichophyton corporis*, *Epidermophyton floccosum* and *Candida* spp.	39	8
Moniliasis of mouth and intertriginous areas	*Candida albicans* and *Candida* spp.	8	1.6
Onychomycosis	*Trichophyton Schoenleinii* and spp.; *Candida albicans*	11	2.4
Ichthyosis hystrix	*Trichophyton* spp.	1	0.2
Erythrasma	*Nocardia minutissima* and *Trichophyton* spp.	1	0.2
Other miscellaneous fungus diseases of the skin	*Trichophyton* spp., *Microsporum* spp., *Candida* spp. *Actinomyces* spp. and *Blastomycetes* spp.	18	6

TABLE 3

Clinical laboratory classification of 381 cases of pyodermias and ulcers not due to fungi

CLINICAL DIAGNOSIS	NO. OF CASES	PERCENTAGE OF TOTAL
Tropical ulcers	160	42
Scabies with pyodermias	57	15
Frambesiform lesions of yaws*	21	6
Furunculoses and acrodermatoses	19	5
Otitis media acutus with involvement of external auditory canal and cellulitis	37	10
Leprosy ulcers	5	1
Impetigo	7	2
Tunga penetrans infestations with pyodermia	5	1
Miscellaneous skin lesions including some venereal diseases	70	16

* These lesions were present in 7 per cent of the cases of yaws.

There were 131 cases of the miscellaneous group with skin lesions not covered in previous tables. In a large percentage of these cases the etiological agents either were not identified or, because of mixed infection, we were not able to determine the most probable primary invaders. Table 4 shows the distribution of some of these diseases according to clinical or working diagnosis.

Some of the patients in these groups had more than one type of dermatological condition.

There were 166 adult or adolescent patients with nonsyphilitic inflammatory conditions of the urogenital system. The vast majority of these 166 patients had Neisserian or *Trichomonas vaginalis* infections. There were 65 females and 101 males in the study.

The clinical conditions in the 65 female adults occurred in the following order:

Trichomonas vaginalis vaginitis	37
Vaginitis with unidentified etiology	12
Cervicitis	24
Pruritus vulvae with or without *Trichomonas* vaginitis	17
Urethritis	16
Salpingitis	8
Others	8

TABLE 4

Distribution of 131 cases of miscellaneous dermatoses, dermatites or other pathological conditions involving the skin

CLINICAL DIAGNOSIS	NO. OF CASES	PERCENTAGE OF TOTAL
Avitaminoses	33	25
Eczematoid dermatitis	11	8
Keloids or hypertrophy of tribal markings	41	16
Varicose veins and telangiectases	9	7
Urticarias and miliarias	10	8
Acnes	18	14
Lichen planus and psoriasis	3	2
Verrucae	7	5
Pemphigus	2	1
Adenolipomatosis	1	1
Dermatalgia	1	1
Ecthyma	1	1
Miscellaneous	8	6

The clinical conditions in the 101 male adults occurred in the following order:

Urethritis—Neisserian	81
Urethritis and/or cystitis of unidentified etiology	17
Strictures of urethra with inflammation or edema	9
Epididymitis	4
Prostatitis	3
Others	3

Special tests

Several special tests were done on selected groups of the Gola tribe, as described below:

Fragility tests: These tests were made on the bloods of 100 men, women and

children to determine whether there was any significant difference between the positives and negatives for plasmodia. No significant difference was observed.

Sicklemia Tests: For field use the Scriver and Waugh (1930) stasis method was used for detecting the sickle-cell traits. These tests were made on 100 children; 19 per cent showed positive sickling traits. Clinical sickle-cell anemia was seen in one case outside of this group during the whole study. It was a fatal case with marked intravascular hemolysis in which the peripheral red blood corpuscle count dropped to less than 1,000,000 per cu. mm. and the hemoglobin to less than 40 per cent. There was a marked splenomegaly accompanying the hemolytic crises. In one family of 5 children, 3 gave positive laboratory sickle-cell trait reactions without any easily recognized clinical symptoms.

Tuberculin patch tests: These tests were done on 100 adolescent children; only 4 per cent were positive.

Blood typings: Blood groupings and biochemical racial indices were done on the same 100 Gola children on which the sickle-cell trait tests were done. The findings showed:

Group O in	74%
Group A in	11%
Group B in	10%
Group AB in	5%
Biochemical racial index in	1.1%
Anti N agglutination in	18%
Anti M agglutination in	36%
Anti NM agglutination in	39%
No agglutination against N or M in	7%*

Sedimentation rates: The simplified Westergren's method was used as described in the Laboratory Manual by Todd and Sanford (1948). Sedimentation rates on 35 women and girls gave an average of 4.2 mm. in one hour, and for 65 men and boys the rate was 4.5 mm. in one hour.

White blood corpuscle counts: The total white blood corpuscle count averaged between 5,000 and 6,000 per cu. mm. for ambulatory individuals. In general the differentials show mild leucopenia of neutrophils, relative lymphocytoses and eosinophilia.

Red blood corpuscle counts: The average total red blood corpuscle count for the same group was 3,850,000 per cu. mm. No case of primary anemia was seen in our series.

Coagulation and bleeding times: The coagulation time for 100 Gola men, women and children (no infants) was 4.6 minutes using the finger puncture blood in 1.5 mm. diameter capillary glass tubes as described by Todd and Sanford (1948), p. 178. The average bleeding time was 2 minutes using the blotting page method of Duke as described by Todd and Sanford pp. 183–184.

Several cases of *macrocytic hypochromatic anemia* and 2 nonfatal cases of *erythoblastosis fetalis* were seen during the study.

* This may be a technical error.

Dental pathology: By the method of rapid oral examination by inspection without the aid of special apparatus, 331 or 11 per cent of the 3,000 individuals examined were found to have some form of dental pathology. Dental caries and periodontoclasia made up the vast majority of the cases in the ratio of 6:1 between caries and periodontoclasia. The number of cavities (caries) varied from 1 to 5 in different individuals. All stages of caries were seen; they ranged from first degree caries to those with nerve root exposed and pulp undergoing putrefaction.

Special attention was given to dental pathological conditions among children of school age. Dental caries was 50 per cent more prevalent among girls than boys in the same age group. Periodontoclasia in varying degrees showed a greater frequency among the boys. Very few of these individuals had ever had any type of professional dental care.

Mortality under 6 years: An analysis of the histories of over 100 Gola women of child-bearing age, in which special attention was given to the number of pregnancies, abortions, miscarriages, stillbirths, infant deaths and deaths among young children, showed that only 20 per cent of the products of conception reached the age of 6 years.

DISCUSSION

The individuals of the Gola tribe are handicapped by medical factors which interfere with the normal physical, mental and intellectual growth and development of the children, and limit the economic and industrial potentials of the adults. While the most easily demonstrated and consistent clinical-laboratory pathological state seen in these handicapped individuals is anemia, the etiological factors responsible for it in the vast majority of cases are protein and vitamin deficient diets, malaria and hookworm infections.

The fact that boys show a higher incidence of malaria and hookworm infections than girls of the same age and district reflects their greater degree of exposure.

The hyperkeratotic lesions of the feet, one of the clinical manifestations of yaws, constitute a distinct economic handicap because the weight-lifting and head and shoulder bearing power of the victims are materially reduced.

The colossal waste of human fecundity, reflected in the fact that only 20 per cent of the products of conception reach 6 years of age, shows the need for a more active and vigorous maternal and infant welfare program. Ignorance, poverty and infectious diseases affecting both parents and offspring are the major factors responsible for this waste of human life.

An analysis of 300 parents and siblings shows the average life expectancy to be about 39 years. This is surely a factor in the small number of degenerative diseases seen in the Gola group. They die before reaching the age when degenerative diseases begin to be manifested.

There is a combination of symptoms among certain native African tribes referred to as the "Open Mole" of the adult. Medical schools, medical journals and standard medical texts either do not mention the condition or do not recognize

it as an entity. The "Open Mole", "Craw Craw" and a few other conditions referred to by the natives as definite diseases do not have known specific etiologies.

The cases of "Open Mole" in this study present a certain similarity of symptoms and physical findings. These were all adults with widened sagittal sutures and easily felt pulsations in the anterior fontanelle regions. All of them showed elevated blood pressures. Some showed signs of increased intracranial pressure such as pupil changes, hyperactivity of the peripheral reflexes, rigidity of the neck, etc. There were areas of tenderness over the head or face. Headache was a common symptom to all. The Kahn tests and malaria smears were negative except in one case. This appears to be a syndrome worthy of further study.

SUMMARY AND CONCLUSION

A sample study of an African tribe relatively uninfluenced by western culture reveals that malaria, treponemiasis and hookworm infections are the three most important diseases in the Gola tribe, with average incidences of 15 per cent, 28 per cent and 62 per cent respectively. Some form of dermatitis or dermatosis was present in 32 per cent of those examined, with mycotic infection, tropical ulcers and pyodermia and scabies making up nearly 80 per cent of these skin lesions. The two most important pathological conditions of the urogenital system were gonorrhea and *Trichomonas vaginalis* vaginitis.

Sicklemia was present in 19 per cent of a group of 100 children in this tribe. The blood picture was otherwise normal except that the differential count showed a relative lymphocytosis and a neutropenia.

Eleven per cent of the group showed some form of dental pathology, with dental caries more prevalent in the girls than in the boys of the same age and district.

The incidence of degenerative diseases in the Gola tribe is comparatively low. A condition called the "Open Mole" by the natives is discussed briefly.

REFERENCES

Ash, J. E., and Spitz, Sophie 1947, Pathology of Tropical Diseases, an Atlas published by W. B. Saunders Co.

Conant, N. F., Martin, D. S., Smith, D. T., Baker, R. D., and Calloway, J. L. 1944, Manual of Clinical Mycology, A National Research Council Publication. Dodge, Carroll William 1935, Medical Mycology, published by C. V. Mosby Co.

Michelson, L., and Wilcox, A. 1940, A rapid thick film blood stain, *Pub. Health Rep.* **55:** 1221.

Poindexter, Hildrus A. 1950, Tropical ulcers, *Arch. Dermat. & Syph.* **62:** 624.

Scriver, J. B., and Waugh, T. R. 1930, Studies on a case of sickle-cell anemia, *Canad. M. A. J.* **23:** 375.

Simmons, James Steven, and Genzkow, Cleon J. 1948, Laboratory Methods of the U. S. Army, published by Lea and Febiger.

Todd, J. C., and Sanford, A. H. 1948, Clinical Diagnosis by Laboratory Methods, published by W. B. Saunders Co.

THE COURSE OF THE BLOOD-INDUCED *PLASMODIUM BERGHEI* INFECTION IN THE MEADOW MOUSE *MICROTUS PENNSYLVANICUS PENNSYLVANICUS* AND CERTAIN OTHER SMALL RODENTS

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In the white mouse *Plasmodium berghei* produces a fulminating type of infection which is almost invariably fatal, but in most other rodents this parasite produces a mild, transient infection followed by periods of latency and recovery. Rodhain (1949, 1951) induced infections in the cotton rat and found that this rodent was easily infected with *Plasmodium berghei* but the resulting infection was of low grade and short-lived. A similar type of infection was described by Ramakrishnan and Prakash (1950, 1951) in the Indian garden squirrel (*Sciurus palmarum*), in the North African rodent (*Meriones shawi*) by Sergent and Poncet (1950), and in the bat (*Roussettus leachi*) by Van Riel (1950).

In the tree rat (*Thamnomys surdaster*) Vincke and van den Bulcke (1949) and in the white rat (*Rattus rattus*) Vincke and van den Bulcke (1949a), Sergent and Poncet (1950), Raffaele and Baldi (1950), Ramakrishnan and Prakash (1950, 1951), Baldi (1950), Galliard and Lapierre (1950, 1951), Mercado and Coatney (1951) described a similar low grade infection; however, in these rodents the primary acute infection was more severe than those mentioned in the paragraph above. In the Palestinian field vole (*Microtus guentheri*) Adler *et al.* (1950) and in the English bank vole (*Clethriomonys glareolus brittanicus*) Bray (1951) observed mild *berghei* infections followed by recovery.

The present investigation deals primarily with the course of the *P. berghei* infection in the American meadow mouse, *Microtus pennsylvanicus pennsylvanicus* plus limited data on this infection in other small rodents.

MATERIALS AND METHODS

The meadow mice employed throughout these investigations were laboratory reared. Due to the relatively small number of animals available at any one time only 4 to 10 specimens, generally females approximately six weeks old, were included in each series.

The animals received intravenously approximately half a million red blood cells parasitized with the KBG 173 strain of *Plasmodium berghei*. A total of 88 mice was inoculated. Starting on the second or third day after inoculation blood smears were made daily during the entire period of the infection. The blood smears were stained with Giemsa. A daily record was kept of the parasitemia and this was expressed as the percentage of red blood cells parasitized rather than as the parasite count per 100 red cells because of the common occurrence of multiple infections. A record was also kept as to the occurrence of segmenters and counts made of the number of merozoites produced per segmenter.

In order to study the effect of the infection on the erythrocyte count, red blood

cell counts were made immediately before inoculation and at intervals during the course of the infection.

In order to determine the total length of the infection in recovered animals, intravenous subinoculations were given to white mice at 2- to 4-week intervals; each recipient received 0.1 cc. of whole blood.

EXPERIMENTAL RESULTS

Although 88 animals were actually inoculated, 7 of them died shortly afterward and were not considered further. The remaining 81 animals became infected; 27 (33.3 per cent) died of the infection, 53 (65.4 per cent) recovered, and one (1.2 per cent) died of an injury on the fourth day of patency.

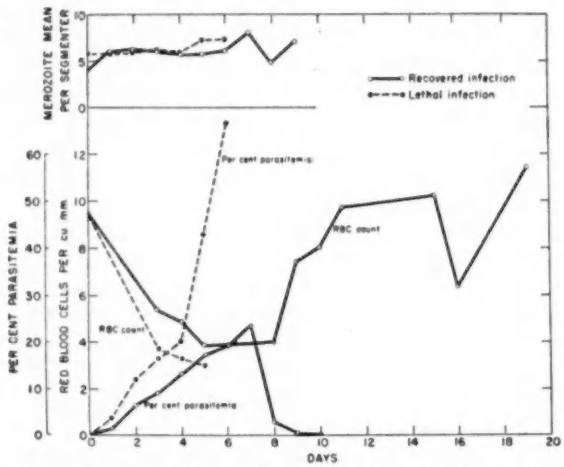

FIG. 1. The average per cent parasitemia, average erythrocyte count, and mean merozoite count per segmenter in lethal and recovered *Plasmodium berghei* infections in American meadow mice during the patent period.

Among the animals which succumbed to the malarial infection the prepatent period varied from 2 to 4 days, average 3.0 ± 0.1. The parasitemia increased progressively but gradually during the first 4 days of patency, but on the fifth and sixth days marked and rapid increases occurred to 43.2 ± 6.2 and 66.5 ± 2.0 per cent respectively (Fig. 1). The peak parasitemia was reached in one to six days, average 3.0 ± 0.4, and death, in 88 per cent of the cases, occurred within 24 hours after the peak; 8 per cent of the deaths occurred 2 days after the maximum parasitemia and 4 per cent died after 3 days. The patent period varied from one to seven days, average 4.1 ± 0.2.

The daily merozoite mean per segmenter varied little during the course of the infection. An initial average count of 5.8 ± 0.5 obtained on the first day of parasitemia remained more or less stable during the first 4 days of patency; on the fifth and sixth days there was a slight increase to 7.4 ± 0.6 and 7.3 ± 3.0 respectively (Fig. 1).

The red blood cell count decreased steadily as the parasitemia increased. From an initial average normal count of 9.5 ± 1.8 million per cu. mm. a marked drop to 3.7 ± 0.9 occurred on the third day of patency. This was followed by small decreases on the fourth and fifth days. The lowest count of 3.1 ± 0.6 million per cu. mm. obtained on the fifth day corresponded more or less with the first rapid increase in the parasitemia.

In the recovered infections the prepatent period varied from 2 to 4 days, average 2.8 ± 0.1. The average daily parasite counts showed a steady increase to 23.6 ± 5.2 per cent on the seventh patent day; this was followed by a marked drop to 2.6 ± 0.8 on the eighth day. This decrease in the parasitemia continued until parasitized cells no longer appeared on the blood smears; a low count of 0.01 per cent occurred on the tenth day. The peak parasitemia was reached in one to six days, average 3.2 ± 0.2, and the first negative smears were obtained in one to seven days following the peak, average 3.4 ± 0.2. The patent period varied from 3 to 11 days, average 6.7 ± 0.3.

As in the lethal infections the merozoite mean per segmenter showed little change during the course of the infection particularly during the first 6 patent days. From an initial value of 4.0 merozoites per segmenter obtained on the first day of parasitemia, the mean number increased slightly to 6.1 ± 0.6 on the sixth day. This was followed by an increase to 8.1 ± 0.9 on the seventh day after which a drop to 4.7 ± 0.3 and an increase to 7.0 ± 1.1 occurred on the eighth and ninth days respectively.

The effect on the red blood cell count was similar to that obtained in the lethal infections during the first 5 patent days but the decrease was less marked. From an initial average normal count of 9.5 ± 1.8 million per cu. mm. the count fell steadily to 3.9 ± 0.4 on the fifth day; thereafter, the red cell count increased steadily as the animals recovered from the parasitemia. A high erythrocyte count of 8.0 ± 1.5 million per cu. mm. which corresponded to a low parasitemia of 0.01 per cent was obtained on the tenth day. Red blood cell counts after the tenth day as shown in the graph refer to cases which were negative on blood smear but were being followed for possible latent infections.

Subinoculations from 44 of the 53 animals which recovered from the acute attack showed that the infection was still present in 9 animals 19 to 51 days following the last postive smear.

COURSE OF THE INFECTION IN OTHER RODENTS

Blood-inoculated infections with the same strain (KBG 173) were studied in 8 other species of rodents: rice rats, *Oryzomys palustris;* pouch mice, *Perognathus penicillatus*, *P. baileyi*, and *P. intermedius;* kangaroo rats, *Dipodomys spectabilis* and *D. merriami;* cotton rats, *Sigmodon hispidus hispidus;* and Egyptian mice, *Acomys cahirinus*. Because of the small number of animals of each species available, detailed data on the course of the infection in each species could not be obtained.

Thirteen rice rats (*Oryzomys palustris*) were inoculated and all became infected. Nine of these animals (69.2 per cent) died as a result of the infection and four (30.8 per cent) recovered. Lethal infections were characterized by an aver-

age patent period of 16.6 days and a peak parasitemia of 25 per cent. Among the recovered animals the average patent period was 53.2 days with a peak parasitemia of 18 per cent. One animal was still infected, as shown by subinoculation, 54 days after the last positive smear.

In *Perognathus penicillatus* the infection resulted in death in 13 out of 15 animals inoculated. In the animals which died of the infection the patent parasitemia averaged 20.5 days with an average peak count of 50 per cent; the recovered animals had an average patent period of 48 days and a peak parasitemia of 44 per cent. When the infection was transferred to *P. baileyi* and *P. intermedius* the mortality rate was 100 per cent. In nine specimens of *P. baileyi* the average patent period was 15.2 days with a peak parasite count of 23.5 per cent. Four specimens of *P. intermedius* exhibited an average patent parasitemia period of 9.25 days and an average peak parasite count of 23.5 per cent.

Three kangaroo rats (*Dipodomys spectabilis*) were inoculated with *P. berghei*. Two of these animals died of the infection and one recovered. The former had an average patent period of 28.5 days and an average peak parasitemia of 4 per cent; the latter exhibited a patent period of 47 days and a peak parasite count of 8 per cent. Two *D. merriami* were also inoculated. One died after 6 days with no evidence of infection; the other died on the 24th day of patency, following a peak count of 65 per cent.

Eighteen Egyptian mice (*Acomys cahirinus*) were inoculated and 10 became infected, but complete data on the infections are available from only 3 animals. Two of these animals died, one on the third day and one on the forty-second day of patent parasitemia. In the latter animal the parasite count was low for over a month but finally rose to 71 per cent shortly before death. In the animal which recovered, the patency continued during 38 days with a peak parasite count of 48 per cent.

The infection in the cotton rat (*Sigmodon hispidus hispidus*) was of low grade and of short duration. Nine animals were inoculated; six became infected and all recovered. The average patent period was 5.5 days with a peak parasite count of 0.5 per cent.

DISCUSSION

In the American meadow mouse (*Microtus p. pennsylvanicus*) blood-inoculated *Plasmodium berghei* produced an infection which manifested itself in two ways; 33.3 per cent of the animals exhibited a progressive parasitemia which resulted in death and 65.4 per cent recovered after a parasitemia which increased gradually to a peak and then decreased rapidly until parasites no longer appeared on smear. Zuckerman *et al.* (1951) working with *M. guentheri* found that 78 per cent of the animals recovered from the infection. In general, the lethal infections in *pennsylvanicus* behaved like those reported for white mice and white rats (Mercado and Coatney, 1951, 1951a), but with a shorter patent period. In white mice and white rats the average periods of patency were 10 to 12 days respectively as against 4 days in *pennsylvanicus*. As in the white mice and white rats a sharp drop in the red blood cell count marked the height of the infection in the American

meadow mouse; the count fell to 3.7 ± 0.9 million per cu. mm. from an initial normal of 9.5 ± 1.8 million per cu. mm. Galliard and Lapierre (1951) reported a similar drop in the white rat.

The change in the average number of merozoites produced per segmenter during the course of the infection was slight when compared with the findings in white mice and white rats (Mercado and Coatney, *loc. cit.*). In white mice the merozoite mean was as high as 8.0 ± 0.4 at the start of the infection, decreased to 5.5 ± 0.1 on the third patent day, and gradually increased to 12.8 ± 0.5 near the terminal stage; in white rats the mean ranged from 5.5 ± 1.6 on the first patent day to 14.1 ± 0.2 on the eighth; the lowest count occurred on the sixteenth day after which it rose to 12.2 ± 1.3 on the eighteenth.

The general pattern of the recovered infections in the white rat (Mercado and Coatney, 1951) and the meadow mouse were similar; however, in the latter the patent period ranged from 3 to 11 days and the decrease in the parasitemia after the peak was uninterrupted and rapid. In the white rat the patent period ranged from 16 to 22 days; the recovery process was more gradual and was interrupted by small increases before smears became negative. The effect on the red blood cell count and on the merozoite mean was also less marked in *M. p. pennsylvanicus* where the red cell count fell to 3.9 ± 0.4 million per cu. mm. on the fifth patent day. In the white rat a count of 0.8 million per cu. mm. was obtained on the twelfth day. The *pennsylvanicus* merozoite mean, low at the beginning of patency, rose to 8.1 ± 0.9 on the seventh day, decreased to 4.7 ± 0.3, and increased to 7.0 ± 1.1 on the eighth and ninth days respectively. In the white rat the merozoite mean ranged from 2 to 3 at the beginning of patency but rose sharply to a count of 16 on the fifth day after which a gradual drop to 4.6 ± 0.5 occurred during the next 11 days. A gradual increase to 9.6 ± 1.0 was observed near the end of patency.

In his investigations of recovered *berghei* infections in the white rat Corradetti (1950) reported a latent period of 28 to 50 days; he also observed that the animals remained immune to reinfection for 205 days. Shorter latent periods of 18 and 10 days respectively were reported by Levaditi and Vaisman (1950) in the white rat and by Ramakrishnan and Prakash (1950) in brown rats. Zuckerman and Yoeli (1951) gave a latent period of 5 to 67 days in *M. guentheri;* our studies showed 19 to 51 days for *M. p. pennsylvanicus* and 54 days in rice rats.

The mechanism whereby certain animals are able to overcome even fulminating infections with *P. berghei* is not known, although several explanations have been put forward. Corradetti (1950) was of the opinion that the spleen or in its absence the reticuloendothelial system of other organs was the key to the process. Rodhain (1949, 1951) showed that removal of the spleen from the recovered cotton rat led to relapse and death or to a chronic infection and subsequent survival; the *berghei* infection, however, was always fatal to those animals inoculated after splenectomy. Van Riel (1950) also reported that splenectomized bats infected with *P. berghei* succumbed to the parasitemia but that nonsplenectomized animals recovered. Similarly, Galliard and Lapierre (1950) noted that splenectomy performed prior to inoculation resulted in heavy infections, but

splenectomy of recovered animals produced only moderate relapses. Fabiani *et al.* (1951) observed that splenectomy performed on white rats a month after recovery resulted in rising parasitemia and death. In their studies on the effect of splenectomy on the course of the *P. berghei* infection in the field vole (*Microtus guentheri*) Zuckerman and Yoeli (1951) concluded that the spleen is essential in establishing immunity and its removal adversely affects the innate and acquired immune mechanisms of the host. More specifically, Vargues *et al.* (1951) attributed the immune state to the production of antibodies which, according to their investigations, appeared on the tenth day of infection, increased until the fifteenth day, and then remained constant during two months; six months after the infection the antibodies were detected in low titer.

Galliard and Lapierre (1950) related the age of the animals to the severity of the infection; in young 50-gram animals the parasitemia was intense and 50 per cent of the rats died; in older 120-gram specimens the mortality rate was only 25 per cent; in 250-gram animals no deaths occurred. Furthermore, the effect of splenectomy was more severe in the young animals but did not seem to influence greatly the course of the infection in the older ones. Deschiens and Lamy (1951) showed that newborn rabbits were easily infected with *P. berghei* and in many cases death ensued, but in animals 15 days old or in adults no infections were produced.

As in most infections the mechanism which permits individuals of certain species to recover from infections of *P. berghei* can only be determined by further investigation.

SUMMARY

Plasmodium berghei in the American meadow mouse (*Microtus pennsylvanicus pennsylvanicus*) was fatal to one-third of the 81 mice experimentally infected. Up to the time of death, the lethal cases showed few significant differences from those which recovered. The peak parasitemia was reached within 6 days, the average being about 3 days in both groups. Death occurred some 24 hours after the peak in all but a few of the lethal cases, but in the animals which recovered, parasitemia continued for three or four more days, when the smears became negative. Subinoculations showed however that in at least 9 of the recovered animals the infection was still present 19 to 51 days following the last positive smear.

The merozoite mean per segmenter increased in both groups during the course of infection from a low of 4 to 7.3 in the lethal cases just before death, and to as high as 8.1 in the animals which recovered, fluctuating to lower levels as the parasitemia disappeared.

The red blood cell count dropped from a normal of 9.5 million per cu. mm. to 3.1 m. in lethal cases, and to 3.9 m. in animals that recovered, but rose again in the latter to 8 m. by the tenth day.

P. berghei was found infective to the rice rat, *Oryzomys palustris;* pouch mice, *Perognathus penicillatus, P. baileyi* and *P. intermedius;* the kangaroo rats, *Dipodomys spectabilis* and *D. merriami;* the Egyptian mouse, *Acomys cahirinus;*

and the cotton rat, *Sigmodon hispidus hispidus*, with varying courses of infection as described.

ACKNOWLEDGMENTS

Thanks are extended to Miss Louise Perkins and Miss Nancy Allen for technical assistance.

REFERENCES

ADLER, S., YOELI, M., AND ZUCKERMAN, A. 1950, Behavior of *Plasmodium berghei* in some rodents, *Nature* **166:** 571.

BALDI, A. 1950, Sul quadro anemico nell' infezione da "*P. berghei*" (Vincke e Lips), *Riv. di Malariol.* **29:** 349–356.

BRAY, R. S. 1951, Course of *P. berghei* infection in the English bank vole, *Clethriomonys glareolus brittanicus*, *Trans. Roy. Soc. Trop. Med. & Hyg.* **44:** 362.

CORRADETTI, A. 1950, Particolari fenomeni immunitari nell' infezione da *Plasmodium berghei*, *Riv. di Parassitologia* **11:** 201–209.

DESCHIENS, R., AND LAMY, L. 1951, Infection expérimentale du lapin par *Plasmodium berghei*, Vincke et Lips 1948, *Bull. Soc. Path. Exot.* **44:** 405–409.

FABIANI, G., VARGUES, R., GRELLET, P., AND CLAUSSE, Mlle. J. 1951, Influence de la splénectomie sur des rats blancs spontanément guéris d'une infection expérimentale à *P. berghei*, *Compt. rend.* Soc. de biol. **145:** 1131–1134.

GALLIARD, H., AND LAPIERRE, J. 1950, Effets de la splénectomie sur l'évolution et les rechutes de l'infection à *Plasmodium berghei* Vincke et Lips chez le rat blanc, *Compt. rend. Soc. de biol.* **144:** 402–403.

GALLIARD, H., AND LAPIERRE, J. 1951, Infection à *Plasmodium berghei* chez le rat blanc, *Bull. Soc. path. exot.* **44:** 185–194.

LEVADITI, C., AND VAISMAN, H. 1950, Histo-pathologie de l'infection provoquée chez la souris par le *Plasmodium berghei* (1) le rat, réservoir de virus, *Bull. Soc. path. exot.* **43:** 693–696.

MERCADO, T. I., AND COATNEY, G. R. 1951, The course of the blood-induced *Plasmodium berghei* infection in white rats, *J. Nat. Malaria Soc.* **10:** 359–365.

MERCADO, T. I., AND COATNEY, G. R. 1951a, The course of the blood-induced *Plasmodium berghei* infection in white mice, *J. Parasitol.* **37:** 479–482.

RAFFAELE, G., AND BALDI, A. 1950, Sulla morfologia e sulla transmissione di "*P. berghei*" (Vincke e Lips), *Riv. di malariol.* **29:** 341–347.

RAMAKRISHNAN, S. P., AND PRAKASH, S. 1950, Studies on *Plasmodium berghei* N. Sp., Vincke and Lips, 1948. II. Morphology, periodicity, and pathogenicity in blood-induced infections in mice, rats, and garden squirrels, *Indian J. Malariology* **4:** 369–375.

RAMAKRISHNAN, S. P., AND PRAKASH, S. 1951, Susceptibility of the Indian garden squirrel (*Sciurus palmarum*) to *Plasmodium berghei* and its asexual periodicity, *Nature* **167:** 533.

VAN RIEL, J. 1950, Le comportement de *Roussettus leachi* vis-à-vis du *Plasmodium berghei*, *Ann. Inst. Pasteur* **79:** 772–776.

RODHAIN, J. 1949, Le comportement du cotton rat vis-à-vis du *Plasmodium berghei* (Vincke et Lips), *Ann. Soc. belge de méd. trop.* **29:** 483–490.

RODHAIN, J. 1951, Le comportement du cotton rat vis-à-vis du *Plasmodium berghei*, *Ann. Soc. belge de méd. trop.* **31:** 289–296.

SERGENT, E., AND PONCET, Mme. A. 1950, De la virulence pour le mérion, rongeur nord-africain, de *Plasmodium berghei*, hemosporidie d'un rongeur de l'afrique centrale, *Arch. Inst. Pasteur d'Algerie* **28:** 323–334.

VARGUES, R., FABIANI, G., AND FULCHRION, G. 1951, Étude sérologique de l'infection expérimentale du rat blanc par *P. berghei*. Variations quantitatives de la sensibilisatrice et de l'alexine an cours de la maladie, *Compt. rend. Soc. de biol.* **145:** 1298–1300.

VINCKE, I. H., AND VAN DEN BULCKE, M. A. 1949, Réaction des *Thomnomys surdaster surdaster* vis-à-vis du *Plasmodium berghei*, Vincke et Lips, *Ann. Soc. belge de méd. trop.* **24:** 545–548.

VINCKE, I. H., AND VAN DEN BULCKE, M. A. 1949a, Réaction des *Rattus rattus* vis-à-vis du *Plasmodium berghei*, Vincke et Lips, *Ann. Soc. belge de méd. trop.* **29:** 537–544.

ZUCKERMAN, A., AND YOELI, M. 1951, The effect of splenectomy on the course of *Plasmodium berghei* infections in *Microtus guentheri*, *J. Infect. Dis.* **89:** 130–142.

THE EFFECTS OF VARIOUS TREATMENT PROCEDURES ON THE METABOLISM OF *TRYPANOSOMA CRUZI* AND ON ITS ABILITY TO SUPPORT GROWTH OF *ENDAMOEBA HISTOLYTICA*

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Among the pathogenic protozoa of man, *Endamoeba histolytica* appears to be unique in its requirement of microbial associates for growth *in vitro*. However, in tissues of the host it may thrive in an environment free of other microörganisms. There has been considerable effort to cultivate the ameba in pure culture, but success has not been achieved. Furthermore, despite observations *in vitro* of the ingestion by the ameba of large numbers of microbial associates, their specific role in promoting growth of amebas remains obscure. Nevertheless, the failure of amebas to grow upon transfer from sterile tissue and other sources to a variety of nutrient media (Cleveland and Sanders, 1930; Rees and Reardon, 1947; Rees *et al.*, 1948) suggests a dependence upon the metabolic activity of associates. The data of the present report provide a basis for further examination of such an hypothesis. Attempts were made to cultivate the ameba in various experimental media containing filtrates, homogenates, supernates and dialysates of *Trypanosoma cruzi* cultures, and with *T. cruzi* after suppression of its metabolic activity by each of the following methods: heat-treatment, freezing and thawing, drying, and exposure to distilled water, hydrochloric acid, and ether.

EXPERIMENTAL PROCEDURES

The F-22 strain of *E. histolytica* and the Culbertson strain of *T. cruzi* were used in all experiments. The effects of each of the media with the exception of dialysate medium on the growth of *E. histolytica* were evaluated in test tubes and in microtubes; the dialysate medium was evaluated only in test tubes. Preparation of the various media was as follows: ameba-trypanosome (A-T) medium, the positive control, in which cultures of the ameba were maintained with untreated *T. cruzi*, was composed of a 7-day-old culture of *T. cruzi* in a trypticase-serum medium, 4 parts, and a saline-sodium thioglycollate solution, 1 part (Phillips, 1951). Each of the experimental media was prepared by the same formula except that the *T. cruzi* cultures were treated before they were utilized in the experimental media by the following methods: filtrates were prepared by passage of the trypanosome cultures through Berkefeld filters of normal porosity. Cell-free supernates were utilized following the removal of *T. cruzi* by centrifugation for 15 minutes at 4,000 r.p.m. The *T. cruzi* homogenates were prepared by transferring the culture material at 5°C. to Mickle vials containing glass beads and exposing the organisms therein to the vibrations of a Mickle electro-magnetic 60 cycle vibrator for 30 minutes (the minimum time required for disintegration of all *T. cruzi*). The dialysate medium was prepared by a method similar to that

used by Tobie and Rees (1948) for the cultivation of *T. cruzi*. Loops of cellophane tubing were suspended into the overlay of diphasic blood-agar medium in 25 x 200 mm. test tubes, and the loops were filled to the level of the surrounding fluid with trypticase-serum-sodium thioglycollate medium. The medium surrounding the loop was inoculated with *T. cruzi* and incubated for 5 days at which time the medium within the loop received an inoculum of approximately 25,000 amebas.

Prior to testing heat-treated *T. cruzi* for amebic growth-promoting properties, the temperature required for immediate inactivation of the trypanosomes was determined. Erlenmeyer flasks of 125 ml. capacity containing 50 ml. of 7-day-old *T. cruzi* cultures were suspended to a depth of 2 inches in a water bath equipped with an electric stirrer. To ensure the uniform distribution of heat in the medium, the flasks were kept in motion by a motor-driven agitator. The temperature within the flasks was recorded at one minute intervals, at which time representative samples of the flagellates were examined microscopically. It was found that a temperature of 48°C. $\pm$ 0.5° resulted in immediate inactivation of the *T. cruzi*. This temperature was obtained within the flask by treatment at water bath temperatures of 48°C. for 10 minutes, 50°C. for 5 minutes, or 60°C. for 1.5 minutes. The criteria for inactivation of the heat-treated organisms, and for all treated *T. cruzi* used in the various experimental media, were the absence of motility and failure to establish cultures when transferred to diphasic blood-agar medium.

Medium containing *T. cruzi* after freezing and thawing was prepared by storing A-T medium for 20 hours at -10°C. followed by storage for 1 hour at 37°C. During this procedure the tubes were sealed with petrolatum to minimize oxygenation of the medium.

The other treatment procedures were employed with concentrations of *T. cruzi* obtained by centrifugation. Drying of *T. cruzi* was accomplished by storage for 24 hours in a desiccator jar evacuated to a pressure of 30 mm. Exposure of the trypanosomes to distilled water, N/25 HCl and to ether was accomplished by adding each in quantities approximating 25 per cent of the volume of removed supernate. Following exposure for 30 minutes at room temperature, the preparations were centrifuged, the distilled water, HCl and ether, respectively, were discarded, and the treated suspensions of organisms were washed three times in approximately 30 volumes of sterile Locke's solution. The residua were then resuspended in their respective supernates.

The inocula of amebas for the test tube experiments were derived from ameba-trypanosome (A-T) cultures. The amebas for inoculation of the microculture tubes were obtained from cultures maintained in a modification of the medium of Shaffer, Ryden, and Frye (1949). In this medium a nutrient liver-infusion broth was substituted for the regular saline component. The liver broth contained the following: Bacto-liver (Difco) 50.0 gm., Tryptone (Difco) 5.0 gm., NaCl 7.0 gm., KCl 0.4 gm., CaCl 0.4 gm., $NaHCO_3$ 0.4 gm., and distilled water 1,000 ml. The microculture tubes and the method of their inoculation with a single, washed trophic ameba have been previously described (Phillips, 1950; Phillips and Rees, 1950).

In order to ascertain whether the treatment procedures had completely suppressed the metabolic activity of *T. cruzi*, each of the media containing the treated organisms was subjected to the following studies: measurements of oxygen uptake were obtained with a Warburg respirometer[1] to determine to what extent the aerobic respiration of the organisms had been inhibited. Furthermore, since there may have been activity by the treated organisms even in the absence of oxygen uptake, the media were evaluated for their efficacy in the reduction of methylene blue and pH values were recorded at various intervals to determine whether there was acid production by treated *T. cruzi*. The Warburg studies utilized the method described by von Brand, Johnson, and Rees (1946) except that 3 ml. samples of the media containing approximately 100 million trypanosomes were utilized in each of the Warburg vessels. The efficacy of the various media in the reduction of methylene blue was based upon the time required to decolorize the dye in the concentration of 1 to 500,000 in test tubes sealed with petrolatum. The pH measurements were made with a Beckman pH meter at intervals of 0, 24, 48, and 72 hours.

EXPERIMENTAL DATA

Results of the studies to ascertain the degree of metabolic inhibition of the treated organisms are summarized in Table 1. These data show that each of the media exhibited some oxygen uptake. However, this was not attributable to the treated organisms since the cell-free supernate medium gave Warburg readings comparable to each of the media containing the treated organisms. Only the medium which contained untreated *T. cruzi* gave readings significantly higher than those of the cell-free supernate or of a trypticase-serum-sodium thioglycollate medium which was not conditioned by the growth of *T. cruzi*. Hence the aerobic metabolism of *T. cruzi* was completely suppressed by each of the treatment procedures. The oxygen uptake of the various media was probably attributable to the presence of serum and sodium thioglycollate.

In contrast to the foregoing results, the methylene blue studies showed some activity by heat-treated *T. cruzi* even though its aerobic metabolism was completely suppressed. In the positive control with untreated *T. cruzi*, methylene blue was reduced in approximately 20 minutes. In the medium containing heat-treated *T. cruzi*, the reduction of the dye required 35 minutes. However, each of the other media containing treated *T. cruzi* required more than twice as long before the dye was reduced; similar results were obtained with the cell-free supernate medium.

The pH determinations, as shown in the table, provide further confirmation of metabolic activity by heat-treated *T. cruzi*. In test tubes containing the heat-treated trypanosome medium there was a significant lowering of pH, 0.35 pH units in 24 hours, whereas in other media with treated *T. cruzi* no changes in pH occurred. The acid production occurred only during the first 24 hours since no significant changes in pH occurred thereafter.

[1] The supervision of the Warburg studies by Dr. Harry D. Baernstein of the Laboratory of Tropical Diseases is gratefully acknowledged.

The effects of the various experimental media on the growth of *E. histolytica* from single cell isolations in microtubes are also summarized in Table 1. These data show that, with one exception, heat-treated *T. cruzi*, inactivation of the associate was accompanied by a complete loss of the amebic growth-promoting properties of the medium. For example, in microtubes containing untreated

TABLE 1

Oxygen uptake, methylene blue reduction, and acid production after various treatments of the T. cruzi *component of ameba-trypanosome medium; effects of treatment on the growth of* E. histolytica

AMEBA-TRYPANOSOME MEDIUM, *T. cruzi* component	TYPE OF ASSAY				ACID PRODUCTION, pH CHANGE[2]			GROWTH OF *E. histolytica* TOTAL COUNT[3]		
	Manometric[1]			Thunberg (mod.)						
	cmm. O_2			Methylene blue reduction time	pH units			Number of amebas		
	1 hr	2 hrs	3 hrs	Minutes	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Untreated *T. cruzi*	34 (21)	58 (28)	72 (32)	20	−0.55	−0.65	−0.72	123	914	2470
Heat-treated *T. cruzi*	20 (19)	27 (25)	31 (29)	35	−0.35	−0.38	−0.37	70	50	22
Homogenate of *T. cruzi*	13 (11)	20 (21)	27 (25)	110	0	0	0	10	5	4
Frozen and thawed *T. cruzi*	14 (11)	26 (22)	29 (25)	120	0	0	0	9	6	3
Dried *T. cruzi*	10 (11)	23 (22)	25 (25)	120	0	0	0	10	7	4
H_2O treated *T. cruzi*	19 (15)	23 (20)	25 (23)	110	0	0	0	10	6	3
HCl treated *T. cruzi*	20 (15)	22 (20)	26 (23)	105	0	0	0	9	5	3
Ether treated *T. cruzi*	14 (15)	20 (20)	26 (23)	120	0	0	0	8	4	2
Filtrate of *T. cruzi* culture	15 (15)	19 (20)	24 (23)	130	0	0	0	9	9	3
Supernate of *T. cruzi* culture	15	20	23	110	0	0	0	8	7	3
Trypticase-serum-sodium thioglycollate medium	22	28	31	90	0	0	0	8	5	2

[1] Values for supernate controls in parentheses.
[2] Change from original pH of 7.4. Changes of 0.02 pH units or less listed as 0.
[3] Total amebas in 10 microtubes from single-cell isolations.

T. cruzi, there was growth of amebas for the entire 72-hour period of observation. In microtubes with heat-treated *T. cruzi*, there was growth of amebas for the first 24 hours of incubation with a decrease in numbers thereafter. However, in microtubes with the various other experimental media there was survival of the ameba for periods beyond 72 hours but not a single instance in which multiplication occurred.

The effects of the various experimental media on the growth of *E. histolytica* in test tubes were as follows: cultures of the ameba with *T. cruzi* after heat-treatment of the flagellates for 5 minutes at 50°C. were established and have been maintained through more than 50 serial transfers at 48-hour intervals. Although growth of the ameba is good, it is somewhat less than that obtained with untreated *T. cruzi*. However, in test tubes with other media containing inactive *T. cruzi* or filtrates, supernates and dialysates of *T. cruzi* cultures, the ameba failed to survive beyond the second serial transfer.

DISCUSSION

The primary significance of the present report lies in its suggestion that *E. histolytica* may be capable of multiplication only when in contact with certain other metabolizing organisms. Support for such an hypothesis is provided in that abundant growth of the ameba occurred in A-T medium with untreated *T. cruzi*, and some growth occurred with heat-treated *T. cruzi* which was immotile and incapable of reproduction but which retained some degree of metabolic activity. However, when active *T. cruzi* was separated from the ameba by a cellophane membrane or removed from the conditioned experimental medium, and when the metabolic activity of *T. cruzi* was more completely inhibited by the treatment procedures, no growth of ameba occurred. These procedures probably did not alter the nutritional qualities of the medium.

The present report emphasizes the suitability of the *T. cruzi* associate for cultural studies of the type described herein. Nevertheless, previous studies with inhibited bacterial associates are of considerable interest. The first demonstration of growth of *E. histolytica* in the absence of viable associates was reported by Jacobs (1947). Cultures of the ameba were maintained for a period of three and one-half to six months in Locke's egg-rice starch or Locke's egg-glucose medium to which heat-treated *Escherichia coli* was added at the time of each serial transfer. Shortly thereafter, in 1948, Shaffer, Ryden, and Frye demonstrated good growth of the ameba with a penicillin-inhibited *Streptobacillus* in liquid medium. Although data relative to the degree of inhibition of the bacterial associates used in these foregoing studies were not given, it is probable that in each instance there was some remaining metabolic activity perhaps comparable to that of the heat-treated *T. cruzi* described herein.

Other cultural studies of the ameba, with heat-treated *T. cruzi* as an associate, were reported by Nakamura and Anderson (1951). Data were included on the effects of the treatment procedures on the aerobic metabolism of the trypanosomes. These investigators concluded that the ability of heat-treated *T. cruzi* to maintain growth of *E. histolytica* was correlated with its ability to maintain aerobic respiration. The present studies do not support this conclusion since amebic growth occurred with *T. cruzi* after complete inhibition of its aerobic metabolism. Although no definite explanation can be given for these differences, the present data were obtained by procedures significantly different from those utilized by the previous investigators. For example, in the present studies, since the objective of the Warburg experiments was to determine if *T. cruzi* which

supported growth of the ameba were capable of consuming oxygen, the oxygen uptake was determined in medium identical to that wherein amebic growth occurred. The use of Warburg vessels provided an environment adequate for such determinations. In the studies by the previous investigators, amebic growth was evaluated in a medium similar to that utilized herein but the heat treatment of the trypanosomes for the Warburg studies and the subsequent measurements of oxygen uptake were carried out in a sucrose-saline solution.

The demonstration of amebic growth with heat-treated *T. cruzi* which exhibited no oxygen uptake is of particular interest since it shows the ability of the ameba to grow in an environment wherein the oxygen content is lowered only by components of the medium. This is in agreement with the findings of Jacobs (1950) in that it shows the improbability that oxygen removal is the primary contribution of the ameba associates. Of equal interest are the data which show that *E. histolytica* was capable of multiplication only when in contact with metabolizing associates. In the presence of untreated *T. cruzi* there was a progressive increase in the number of amebas throughout the entire 72-hour period of incubation. When associated with *T. cruzi*, the metabolism of which had been partially suppressed by heat treatment, there was a reduced rate of amebic growth for 24 hours of incubation and no growth thereafter. This shows a correlation with the period during which heat-treated *T. cruzi* exhibited some metabolic activity as indicated by acid production. Although the ameba ingested large numbers of intact, nonmetabolizing associates, it failed to multiply, thus indicating a requirement of more than the source of food which is provided by the associate cell *per se*. Untreated trypanosomes have exhibited motility for periods up to three hours within cytoplasmic vacuoles of the ameba. Hence it is not inconceivable that the necessary factor(s) may be supplied within the amebic cell.

It is also reasonable, on the basis of these results and on the results of others working with bacteria, to postulate that the necessary growth factor(s) for the ameba is (are) supplied in very small amount as a result of the *continuous* metabolic activity of the associates, whether trypanosomes or bacteria, and are never present in sufficient quantity to provide more than brief support for the amebas when the associates are rendered inactive.

SUMMARY

Ten experimental media, each of which contained products of *T. cruzi* metabolism and some of which contained, in addition, *T. cruzi* rendered immotile and nonviable by various procedures, were evaluated for their ability to maintain growth of *E. histolytica*. Other studies of the media, paralleling the foregoing, provided comparative data relative to their oxygen uptake and their efficacy in the reduction of methylene blue. The media were also studied for evidence of acid production by treated organisms. The resultant data showed that abundant growth of ameba occurred with untreated *T. cruzi*, and some growth occurred with treated *T. cruzi* that exhibited no oxygen uptake, were immotile and incapable of reproduction, but which exhibited activity in the reduction of methylene blue and produced acid. However, when *T. cruzi* were separated from the

ameba by a cellophane membrane or removed from the conditioned medium, and when the metabolic activity of *T. cruzi* was completely inhibited, no growth of the ameba occurred. The data suggest the dependence of *E. histolytica* on the metabolism of its associates and indicate thereby the probable area wherein lies the identity of the contribution by the associates to growth of the ameba.

REFERENCES

VON BRAND, T., JOHNSON, E. M., AND REES, C. W. 1946, Observations on the respiration of *Trypanosoma cruzi* in culture, *J. Gen. Physiol.* **30:** 163–175.

CLEVELAND, L. R., AND SANDERS, E. P. 1930, The production of bacteria-free amoebic abscesses in the liver of cats and observations on the amoebae in various media with and without bacteria, *Science* **72:** 149–151.

JACOBS, L. 1947, The elimination of viable bacteria from cultures of *Endamoeba histolytica* and the subsequent maintenance of such cultures, *Am. J. Hyg.* **46:** 172–176.

JACOBS, L. 1950, Oxidation-reduction potentials in the cultivation of *Endamoeba histolytica*, *Am. J. Trop. Med.* **30:** 803–815.

NAKAMURA, M., AND ANDERSON, H. H. 1951, Effect of heat-treatment on the respiration of *Trypanosoma cruzi* used for the cultivation of *Endamoeba histolytica*, *Am. J. Trop. Med.* **31:** 438–441.

PHILLIPS, B. P. 1950, Cultivation of *Endamoeba histolytica* with *Trypanosoma cruzi*, *Science* **111:** 8–9.

PHILLIPS, B. P., AND REES, C. W. 1950, The growth of *Endamoeba histolytica* with live and heat-treated *Trypanosoma cruzi*, *Am. J. Trop. Med.* **30:** 185–191.

PHILLIPS, B. P. 1951, Comparative effects of certain species of Trypanosomidae on the growth of *Endamoeba histolytica* in the absence of bacteria, *Am. J. Trop. Med.*, **31:** 290–294.

REES, C. W., AND REARDON, L. V. 1947, Studies on the growth requirements of metacystic *Endamoeba histolytica*, *J. Parasitol.* **33:** Section 2: 21. (Abstract.)

REES, C. W., REARDON, L. V., AND BAERNSTEIN, H. D. 1948, A biochemical approach to the problem of cultivating *Endamoeba histolytica* without bacteria, *J. Parasitol.* **34:** Section 2: 11–12. (Abstract.)

SHAFFER, J. G., RYDEN, F. W., AND FRYE, W. W. 1948, Studies on the growth requirements of *Endamoeba histolytica*. III. The growth and multiplication of two strains of *E. histolytica* in a transparent medium without the addition of rice flour or other particulate matter and without demonstrable bacterial growth, *Am. J. Hyg.* **47:** 345–350.

SHAFFER, J. G., RYDEN, F. W., AND FRYE, W. W. 1949, Studies on the growth requirements of *Endamoeba histolytica*. IV. Further observations on the cultivation of *E. histolytica* and other intestinal protozoa in a clear medium without demonstrable bacterial multiplication. Some modifications and simplifications of the medium, *Am. J. Hyg.* **49:** 127–133.

TOBIE, E. J., AND REES, C. W. 1948, The cultivation of *Trypanosoma cruzi* in dialysate medium, *J. Parasitol.* **34:** 162–163.

THE RELATIVE ACTIVITY OF THE COMMON SULFONAMIDES AGAINST EXPERIMENTAL TOXOPLASMOSIS IN THE MOUSE

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The fact that some of the sulfonamides can repress, or in some instances cure, *Toxoplasma* infections in mice has been established by a number of investigators. Although this fact is known, sufficient data are not available to allow one to select a specific sulfonamide as the drug of choice in toxoplasmosis. The primary purpose of the present investigation was to compare the commonly available sulfonamides under identical conditions in order to determine which drug has the greatest therapeutic activity. Secondarily, the action of sulfadiazine was explored more thoroughly in order to determine under what conditions cure can be obtained, and to establish a base line for comparison with other active drug groups. The drugs utilized in this study were sulfadiazine, sulfamerazine, sulfamethazine, sulfathiazole, sulfapyridine, and sulfanilamide.

The activity of sulfonamides against toxoplasmosis was first demonstrated by Sabin and Warren (1942) who used sulfanilamide, sulfapyridine, and sulfathiazole in a series of experiments. Sulfanilamide, administered as a one per cent diet suspension for 13 days after the intraperitoneal inoculation of varying dilutions of *Toxoplasma* organisms in a suspension of infected brain tissue, caused delay in death in mice given the larger infective inocula and indefinite survival in the case of some of the mice given small inocula. Sulfapyridine and sulfathiazole administered in an identical manner produced indefinite survival in most of the animals given small inocula and delayed death until after the treatment period in the mice given large inocula. Continuing the drug for up to 45 days produced more survivors but the mice inoculated with the largest number of organisms still succumbed to toxoplasmosis after the discontinuation of treatment. In another experiment with sulfathiazole, mice treated on the second day after inoculation survived in significant numbers but mice treated on the fourth day or later all died.

Biocca and Pasqualin (1942) confirmed the fact that the sulfonamides are active against toxoplasmosis. They used sulfanilamide, sulfapyridine and sulfathiazole in oil suspensions administered subcutaneously to mice, at a dosage of one gram per kilogram, immediately after inoculation and on the third and sixth day thereafter. Their mice were inoculated with a suspension of infected liver and spleen tissue injected intraperitoneally. Their controls died in eleven days or less, but the death of the sulfanilamide treated mice was delayed for some days. One of four mice treated with sulfapyridine survived indefinitely, and three of four mice treated with sulfathiazole appeared cured.

In 1943, Biocca described additional experiments employing 0.5 gram per kilogram dosages of sulfapyridine and sulfathiazole in oil suspension administered

on the second, fourth and sixth day after intraperitoneal inoculation of infected liver and spleen suspension. In these experiments sulfapyridine produced survival for an average of 12.6 days as compared with 6.4 for the controls; sulfathiazole allowed the mice to survive for an average of 12.0 days. Biocca (1944) stated that a dosage of 10 grams per kilogram of the sulfa drugs was necessary for cure of toxoplasmosis but presented no additional data in this paper.

Weinman and Berne (1944) treated mice with sulfapyridine and obtained about 95 per cent survival when treatment was initiated on the fifth day after inoculation (controls died in 15 days). These investigators used a dosage of approximately one gram per kilogram per day in two doses continuing for four to six days, the drug being administered subcutaneously in oil suspension or as sodium sulfapyridine injected intraperitoneally. If similar treatment was delayed until 10 or more days after inoculation survival was poor although death of some mice was delayed. The same authors used sulfathiazole in oil suspension (same dosage), but treatment with this drug was delayed until 10 or more days after inoculation and survival was poor. They also used sodium sulfadiazine in one small experiment with good survival. Weinman and Berne produced evidence that treatment did not sterilize the infection in their experiments but produced a carrier state.

Biocca and Nobrega (1946) used sulfadiazine and sulfathiazole in mouse toxoplasmosis, administering a single 0.5 gram per kilogram dose of each drug in oil suspension subcutaneously on the third day after inoculation with a suspension of infected liver and spleen tissue. Seven of 10 mice treated with sulfadiazine survived indefinitely and three of 10 given sulfathiazole did likewise. Controls died on the sixth to eighth day.

Summers (1947) used sodium sulfathiazole in experiments on the effects of *p*-aminobenzoic acid (PABA) on sulfonamide treatment. He found that dosages as low as 80 mg. per cent in diet protected mice during the treatment period but he did not give data on indefinite survival. PABA antagonized the effect of sodium sulfathiazole as might be expected. Summers (1949) again used sodium sulfathiazole (100 mg. per cent in diet on days two through nine, or about 0.2 g. per kg. per day). Mice given inocula of varying size were protected through the treatment period but none survived indefinitely. Sulfamerazine in similar experiments was administered in the diet at the same dosage on days one through nine. Most of the mice survived the treatment period but died before the 26th day after inoculation.

Van Thiel (1950) used sulfadiazine, sulfamerazine, sulfamethazine, sulfathiazole, and sulfapyridine in molecularly equivalent doses. Using sulfadiazine (about 0.33 g. per kg. per day in three doses) in a dilute NaOH solution injected subcutaneously on days 1 through 14, almost all animals survived indefinitely whether the inoculation was intraperitoneal or intracerebral. All survived also when the number of organisms was varied by dilution of the infected brain tissue suspension. Nearly the same results were obtained with sulfamerazine (called percoccide in his paper) and sulfamethazine. Sulfathiazole cured mice inoculated intraperitoneally but mice inoculated intracerebrally died. Sulfapyridine cured

most mice inoculated intraperitoneally but not those inoculated intracerebrally. Controls died in from 5 to 11 days. Most of the experiments involved only small numbers of mice.

Gingrich and Darrow (1951) administered sulfadiazine in the diet for five days to a small number of mice inoculated intraperitoneally with 0.2 ml. of a 1/40 dilution of peritoneal exudate at a rate of 0.8 to 1.2 grams per kilogram per day. Death was delayed until the 22nd day on the average; whereas, the controls died in about seven days. When an inoculum of 250 *Toxoplasma* organisms was used, about half the mice survived indefinitely. In this last case controls died about the eleventh day after inoculation.

Cross (1951) stated that sulfadiazine was "ineffective for therapy" but presented no data to support her conclusion, which is quite different from the conclusion of the other investigators who have tried the compound.

From the above short review it can be seen that most observers have found the common sulfonamides to be active against mouse toxoplasmosis, but the degree of efficiency reported varies greatly from observer to observer. This variation is so great as to suggest strongly the existence of strains varying in their susceptibility to sulfonamides. None of the investigators have adequately tested all of the common sulfonamides under uniform conditions and none have attempted to compare them by determining dose-effect relationships and median effective dosages.

MATERIAL AND METHODS

The present series of experiments utilized a strain of *Toxoplasma gondii* isolated from the wild Norway rat in Memphis (Eyles, 1952). This strain produces uniformly fatal infections in mice, the time to death varying with the number of organisms inoculated. It is maintained by twice-weekly intraperitoneal transfer of 0.05 to 0.3 cc. of a 1:10 saline suspension of peritoneal exudate from infected mice.

Young mice of the NIH general purpose strain, weighing about 20 grams, were used in the experiments. Infected mice were kept three to a mouse jar in an air-conditioned animal room. Clean mice kept as controls died at a rate of 12.5 per cent per two week period during the time covered by these experiments.

For the comparative tests mice were inoculated intraperitoneally with 20,000 *Toxoplasma* organisms secured by diluting peritoneal exudate with physiological saline. Counts were made with a hemacytometer. Exudate was pooled from five different mice in order to eliminate variations in the resulting infections. Most mice inoculated as above died in 6 to 8 days if not treated and no animals survived for 10 days. Results were very uniform from experiment to experiment. The counted inoculum was used with the realization that some intracellular organisms would not be counted; however, the uniformity gained by the use of the measured inoculum made comparisons between experiments more reliable. Larger or smaller inocula were used in additional experiments as indicated in the text. It has been determined experimentally that this inoculum is the equivalent of about 1000 median lethal doses of *Toxoplasma* organisms.

Drugs were started immediately after inoculation. They were administered in a pulverized diet produced by grinding "Friskies" dog cubes (Albers Milling Company) or Purina laboratory chow. Feeders similar to those described by Strong (1950) were used and the daily food consumption of at least one mouse jar from each drug regimen was measured. The regimens were continued for 14 days or until death except for special experiments involving longer periods of administration. Throughout this paper the dosages are given as percentages of the diet. Conversion to per kilogram dosage can be calculated on the basis of four grams of diet per mouse per day.

In order to estimate the activity of the different drugs the median effective dose (MED) was determined. Since none of the regimens consistently cured a large percentage of the mice, the effective dose could not be defined as the curative dose. Instead, the median effective dose was defined as the dose which permitted survival of half of the animals for 10 or more days. The methods of Litchfield and Wilcoxon (1949) were used in calculating the MED. The ten day time interval was used because all control mice are dead by this time and, in the case of drugs in short supply with which comparison must also be made, the shorter time interval (as compared with the 14 day treatment period) is of advantage.

In a typical experiment five groups of nine mice each were used with five geometric descending drug dosages. In each instance, infected mice were maintained as untreated controls, and clean mice were maintained to check on mortality from causes other than toxoplasmosis. Some of the experiments were repeated, and the number of mice to which each regimen was administered is shown in the tabulations which follow.

A limited number of blood sulfonamide levels were run through the courtesy of Dr. Edward Josephson of the National Institutes of Health, Bethesda, Maryland, to whom acknowledgement and thanks are due.

RESULTS

A comparison of the median effective dosages of the sulfonamides: The dose-effect relationships for the different sulfonamides, utilizing an inoculum of 20,000 *Toxoplasma* organisms, are shown in Table 1. The MED's along with their confidence limits, are also presented. From the table it is obvious that, gram for gram, sulfadiazine and the closely related compounds, sulfamerazine and sulfamethazine, are more effective against toxoplasmosis than the other three sulfonamides. Following these compounds in effectiveness are sulfathiazole, sulfapyridine, and sulfanilamide. This immediately suggests that the pyrimidine compounds, sulfadiazine and its congeners, are the drugs of choice against toxoplasmosis in mice as their comparative toxicity is low.

The small differences in the MED among the three most effective drugs are insignificant; however, all three are significantly superior to sulfathiazole, and sulfathiazole in turn is significantly superior to sulfanilamide. Even though the data for sulfapyridine are heterogeneous, it would appear to be intermediate in activity between sulfathiazole and sulfanilamide and is definitely inferior to the three pyrimidine derivatives. If the relative effects of the various sulfonamides

be considered on a mole for mole rather than a gram for gram basis, the conclusions are not changed; based on gram for gram comparison the ordinal rank of the drugs is unaltered.

Blood levels were determined on mice given the MED of the different compounds for four days (except sulfathiazole, the sample of which was lost in the mail), in order to determine if the activity was proportional to blood level (Table

TABLE 1

Dose-effect data for six sulfonamides

DOSAGE LEVELS (PER CENT IN DIET)	PROPORTION OF TREATED MICE SURVIVING FOR 10 DAYS OR MORE*					
	Sulfadiazine	Sulfamerazine	Sulfamethazine	Sulfathiazole	Sulfapyridine	Sulfanilamide
2.0						78% (9)
1.0					44% (9)	67% (9)
0.5	†			67% (9)	61% (18)	44% (9)
0.25	†			67% (9)	0% (18)	44% (9)
0.125	†	†	†	33% (9)		
0.063	63% (54)	71% (18)	56% (18)	22% (9)		
0.031	33% (45)	56% (18)	56% (18)	0% (9)		
0.016	24% (45)	6% (18)	28% (18)			
0.008	0% (9)	0% (9)	0% (9)			
MED (% in diet)	0.040%	0.039%	0.036%	0.190%	0.450%	0.600%
95% Confidence limits	0.051%	0.063%	0.061%	0.053%		1.140%
	0.032%	0.028%	0.021%	0.102%		0.316%

* Numeral in parentheses represents number of mice in group.

† Drug at maximum effect at these levels; these points not used in calculating dose-effect curves.

TABLE 2

Blood sulfonamide levels in mg. per 100 cc. for mice receiving the MED of each drug (except sulfathiazole)

	FREE DRUG	ACETYLATED DRUG	TOTAL DRUG
Sulfadiazine, 40 mg. per cent	2.0	0.4	2.4
Sulfamerazine 39 mg. per cent	3.9	1.0	4.9
Sulfamethazine 36 mg. per cent	2.0	0.7	2.7
Sulfapyridine 450 mg. per cent	2.4	1.7	4.1
Sulfanilamide 600 mg. per cent	12.9	2.6	15.5

2). It is obvious that the poor effect of sulfanilamide is not due to low drug level, but in the case of the other sulfonamides the single sample does not show significant difference in level between the drugs. It is also obvious that the levels resulting from the administration of the MED of sulfadiazine are well below those attainable in the human host.

Table 1 also illustrates a phenomenon observed repeatedly in these experiments, that increasing the drug dosage above the MED soon results in a regimen

giving maximum but not 100 per cent effect. For instance, despite the fact that the MED of sulfadiazine is much below the toxic level, increases in amount administered up to 500 mg. per cent in the diet produced survival of only about two-thirds of the treated mice for 10 days. The reason for this effect is not clear. It is possible that mortality due to extraneous causes is increased due to the illness of the mice. Heterogeneity of the mouse strain is also a possibility.

Possible curative action of sulfadiazine: The foregoing section of this paper demonstrates that mice treated with sulfadiazine survived in significant numbers for 10 days. Table 3 presents data on survival for the 10 day period and for 42 days (including the 14 day treatment period). Mortality due to relapse during the period between the 10th and the 42nd day was considerable, but about 40 per cent of the animals which survived 10 days continued to live for 42 if the dosage was as high as 63 mg. per cent of drug in the diet. Furthermore, the data demonstrate that increasing the sulfadiazine dosage over this figure did not result in significantly higher proportions surviving.

The working hypothesis that animals which survived for 42 days were cured required examination. In order to determine if the surviving animals were free from *Toxoplasma* organisms on the 42nd day, brain and liver tissue from some of the survivors was ground and suspended in physiological saline and injected into clean mice (two clean mice per original survivor). These attempts are summarized in the last column of table 3 and it can be seen that, although some of the animals still had viable parasites, most appeared to be free of *Toxoplasma* Confirmatory third blind passages were carried out in the majority of these tests.

Experiments were also performed to determine if the recovered mice were susceptible to reinfection with *Toxoplasma*, or if immunity had been acquired during the treatment period. A total of eight mice were challenged, as outlined in Table 3, with an intraperitoneal inoculum of 20,000 *Toxoplasma* organisms. All died of the disease, mostly from five to seven days after inoculation; however, in one case a mouse survived for 10 days before succumbing.

Effect on survival of varying the size of the inoculum: The previous data were from experiments using a standardized inoculum of 20,000 *Toxoplasma*. Table 4 presents survival data from experiments in which the size of the inoculum was varied 1000-fold, from 2,000,000 to 2,000 organisms. It would appear from these data that mice given smaller inocula and treated, survived for 10 days more frequently and were cured more frequently than mice given the larger number of organisms. This tendency is not counteracted by giving larger amounts of drug; however, some apparent cures resulted from the higher dosages even when the inoculum was 2,000,000 *Toxoplasma* organisms.

Effect of continuing treatment for longer than 14 days: Two experiments were conducted in which the drug was administered for 42 instead of 14 days. Survival data are presented in Table 5. Although a slightly greater proportion of mice survived 42 days in the long-treated groups, the difference is not statistically significant.

Surprisingly, mice in the long-treated group in five instances relapsed and

TABLE 3

Survival of mice after sulfadizine treatment. Inoculum 20,000 Toxoplasma *organisms*

DOSAGE (MG. PER CENT IN DIET)	NUMBER MICE INOCULATED	NUMBER SURVIVING 10 DAYS	NUMBER SURVIVING 42 DAYS	RESULTS OF SUBINOCULATION OR CHALLENGE OF MICE SURVIVING 42 DAYS
500	33	22 (67%)	5 (15%)	Tissues from 4 mice subinoculated (two passages)—no recipients developed toxoplasmosis. One mouse challenged with 20,000 *Toxoplasma*—died in 5 days from toxoplasmosis.
250	83	56 (67%)	22 (27%)	Tissues from 9 mice subinoculated (two passages in all but one case)—two groups of recipients developed toxoplasmosis. Four mice challenged with 20,000 *Toxoplasma*—all died of toxoplasmosis in 5 to 7 days.
125	59	38 (64%)	15 (25%)	Tissues from 9 mice subinoculated (two passages)—no recipients developed toxoplasmosis. Three mice challenged with 20,000 *Toxoplasma*—all died of toxoplasmosis in 5 to 7 days.
63–80	68	39 (57%)	19 (28%)	Tissues from 10 mice subinoculated (two passages in all but 3 cases)—one group of recipients developed toxoplasmosis. Two mice challenged with 20,000 *Toxoplasma*—both died of toxoplasmosis in 6 to 10 days.
31–40	60	22 (37%)	1 (2%)	No data.
16–20	36	8 (22%)	0	
8–10	18	0	0	

TABLE 4

Effect of varying the size of the inoculum on survival for 10 days and for 42 days. Nine or more mice represented by each percentage estimate

SIZE OF INOCULUM	SULFADIAZINE DOSAGE (MG. PER CENT IN DIET)					
	500	250	125	63	31	no drugs
Per cent mice surviving 10 days						
2,000	89	100	100	100	—	0
20,000	67	67	64	56	31	0
200,000	44	56	22	22	33	0
2,000,000	44	56	33	44	11	0
Per cent mice surviving 42 days						
2,000	78	56	33	67	—	0
20,000	15	27	25	27	2	0
200,000	33	33	11	11	0	0
2,000,000	11	22	11	0	0	0

died while still being treated. This occurred twice in the 31 mg. per cent groups (26th and 29th days); twice in the 125 mg. per cent groups (23rd and 41st day); and once in the 250 mg. per cent group (37th day). These mice had peritoneal exudate containing copious numbers of *Toxoplasma* compared with the negative findings in mice dying during the first two weeks. This leads one to suspect the development of drug resistance. An effort to isolate a resistant strain failed, but further efforts are planned.

TABLE 5

Effect of continuing sulfadiazine treatment for 42 days on survival

DOSAGE (MG. PER CENT IN DIET)	14 DAY SULFADIAZINE REGIMEN			42 DAY SULFADIAZINE REGIMEN		
	No. inoc.	Survived 10 days	Survived 42 days	No. inoc.	Survived 10 days	Survived 42 days
250	83	56 (67%)	22 (27%)	18	12 (67%)	4 (22%)
125	59	38 (64%)	15 (25%)	18	13 (72%)	8 (44%)
63	59	33 (56%)	16 (27%)	18	13 (72%)	7 (39%)
31	51	16 (31%)	1 (2%)	18	6 (33%)	0 (0%)
16	27	5 (19%)	0 (0%)	9	1 (11%)	0 (0%)

DISCUSSION

The purpose of this series of experiments was to select the best of the commonly used sulfonamides. It seems clear that in mice the pyrimidine derivatives, sulfadiazine, sulfamethazine and sulfamerazine, are about equally effective. There seems no reason to suspect that any other order of effectiveness for the sulfonamide group as a whole would hold in other animals including man. On the other hand, the fact that cures were obtained in some cases in the mouse does not mean that cure would take place in other animals, but since experiments cannot be done in man, animal work must provide the basis for therapy. It should be pointed out that, contrary to the situation in man, the mouse is very susceptible to toxoplasmosis and is able to acquire little or no immunity to it even when the infection is held in check with drugs. It seems reasonable to postulate that in man or other animals the immune process might play a part and render the drugs more effective than in mice. Experiments with other animals, particularly those responding to toxoplasmosis in a manner more like that of man, are indicated.

When considering these drugs for the treatment of toxoplasmosis in man it is encouraging to find that the blood levels at which the compounds have effect in mice are well below those safely attainable in man. Further work on this subject in mice and in other animals is needed. The evidence thus far obtained indicates that increasing the sulfonamide dosage (and, as would follow, the blood level) results in a maximal effect much short of 100 per cent survival. The reason for this is not clear at present.

The introductory historical paragraphs called attention to the fact that there may well be strains of *Toxoplasma* differing in their susceptibility to sulfonamides.

It is the impression of the authors that the strain at hand is much more difficult to repress and cure than many of those described in the literature. A wider experience with the various strains of *Toxoplasma* is needed, and it would be particularly advantageous to do chemotherapeutic work on a series of them under identical conditions.

Work now in progress at this laboratory (which will be the subject of another communication) indicates that sulfadiazine may be of interest not only because of its own considerable activity but because of the fact that it acts strongly in a synergistic manner with 2:4′-diamino-5-(4′-chlorophenyl)-6-ethylpyrimidine, which is also active alone (Eyles and Coleman, 1952).

SUMMARY

Defining the effective dose as the dose producing 10-day survival of mice inoculated with 20,000 *Toxoplasma* organisms, median effective doses of the common sulfonamides were measured under uniform conditions, as follows:

Sulfadiazine	0.040 per cent in the diet
Sulfamerazine	0.039 per cent in the diet
Sulfamethazine	0.036 per cent in the diet
Sulfathiazole	0.190 per cent in the diet
Sulfapyridine	0.450 per cent in the diet
Sulfanilamide	0.600 per cent in the diet

Since the pyrimidine derivatives, sulfadiazine, sulfamerazine and sulfamethazine, had the smallest effective dosages and are known to be the least toxic of the sulfonamides tested, it was concluded that these drugs are the most efficient antitoxoplasmic agents.

Although MED's were low, increasing the amount of drug administered did not result in all animals surviving. With the highest dosages only about two-thirds survived 10 days.

Blood chemistry studies showed that sulfadiazine, sulfamerazine, and sulfamethazine are effective in mice at blood levels much below those safely attainable in man.

About one-fourth of the mice given a dosage of 0.063 per cent or more sulfadiazine in the diet survived for 42 days. A number of these animals were tested to see if cure (freedom from parasites) had been effected. In the majority of cases tissues from the surviving mice produced no infection when injected into clean recipient mice. Other surviving animals were reinoculated with *Toxoplasma* organisms and all of these proved susceptible and most died from five to seven days later.

LITERATURE CITED

Biocca, E. 1943, Quimioterapia sulfonica da toxoplasmose, *Arq. de biol.* (São Paulo) **27**: 7–10.

Biocca, E. 1944, Toxoplasmose e seu tratamento quimioterapico, *Rev. Brasil. med.* **1**: 380–382.

Biocca, E. and Nobrega, P. 1946, Sobre a quimioterapia da toxoplasmose, *Arq. de biol.* (São Paulo) **30**: 63–66.

BIOCCA, E., AND PASQUALIN, R. 1942, A ação terapeutica de alguns compostos sulfanilamidicos na infeção experimental per toxoplasma, *Arq. de biol.* (São Paulo) **26:** 107–109.

CROSS, J. B. 1951, Diasone and promin as therapeutic agents in experimental toxoplasmosis, *Proc. Soc. Exper. Biol. & Med.* **76:** 548–551.

EYLES, D. E. 1952, Toxoplasma in the Norway rat, *J. Parasitol.* **38:** 226–229.

EYLES, D. E., AND COLEMAN, N. 1952, Tests of 2:4-diaminopyrimidines on toxoplasmosis, *Pub. Health Rep.* **67:** 249–252.

GINGRICH, W. D., AND DARROW, E. M. 1951, The effect of endochin on experimental toxoplasmosis, *Am. J. Trop. Med.* **31:** 12–17.

LITCHFIELD, J. T., JR., AND WILCOXON, F. 1949, A simplified method of evaluating dose-effect experiments, *J. Pharm. & Exp. Therap.* **95:** 99–113.

SABIN, A. B., AND WARREN, J. 1942, Therapeutic effectiveness of certain sulfonamides on infection by an intracellular protozoan (Toxoplasma), *Proc. Soc. Exp. Biol. & Med.* **51:** 19–23.

STRONG, L. C. 1950, The care of experimental mice, in Farris, E. J., Ed., The care and breeding of laboratory animals. John Wiley and Sons, Inc., New York.

SUMMERS, W. A. 1947, Antagonism of sulfonamide inhibition by para-aminobenzoic acid and folic acid in *Toxoplasma* infected mice, *Proc. Soc. Exp. Biol. & Med.* **66:** 509–511.

SUMMERS, W. A. 1949, The effects of oral administration of Aureomycin, sulfathiazole, sulfamerazine, and 4,4′-diaminodiphenylsulfone on toxoplasmosis in mice, *Am. J. Trop. Med.* **29:** 889–894.

VAN THIEL, P. H. 1950, Treatment of experimentally induced toxoplasmosis with sulphonamides, arsenic compounds and antimalarial drugs, *Doc. Neerl. Ind. de Morb. Trop.* **2:** 51–58.

WEINMAN, D., AND BERNE, R. 1944, Therapeutic cure of acute experimental toxoplasmosis in animals, *J.A.M.A.* **124:** 6–8.

ANTIBIOTICS IN THE TREATMENT OF TOXOPLASMOSIS

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Many conflicting reports on the effect of the modern antibiotics on toxoplasmosis have appeared in the literature. Lack of uniformity in the method of testing for activity may have given rise to some of the discrepant observations. This report concerns a series of uniform tests of the broad spectrum antibiotics aureomycin, chloramphenicol, terramycin and fumagillin[1] against experimental mouse toxoplasmosis. These tests resulted in an estimate of the relative as well as the absolute effect of the four drugs.

In addition to the above, less detailed tests were made of other antibiotics, these being included in a series of drugs screened by this laboratory for antitoxoplasmic activity.

Aureomycin was first reported to be active against *Toxoplasma* infections in mice by Summers (1949). He used dosages of from 50 to 200 mg. per cent of the antibiotic in the diet from two days prior to or from the day of inoculation until nine or 10 days after. Mice were inoculated intraperitoneally with 0.2 cc. of peritoneal exudate from infected mice diluted 10^1, 10^2, 10^3, or 10^4 times. When the more dilute inocula were given all animals survived the treatment period with all regimens. Most of the mice given the larger inocula also lived through the treatment period but all mice of all groups had died by the end of the 26th day after inoculation.

Thalhammer *et al.* (1951) also used aureomycin in mice, administering it subcutaneously in dosages varying from 1.35 mg. to 5 mg. per mouse. When 1.35 mg. of the drug was given t.i.d. on the second and b.i.d. on the third through the fifth day, life was prolonged in the treated group but all animals were dead by the end of the 19th day. In a second experiment the larger dosage was used on the second through fifth and again on the 17th through 20th day, but, although life was prolonged, all mice were dead by the 22nd day.

Steen (1950) inoculated mice intraperitoneally with 0.1 cc. of a 10^3 dilution of peritoneal exudate, then treated them with intramuscular injections of aureomycin of 0.1 mg. q.i.d. at varying times with relation to the time of inoculation. When treatment was instituted either two hours before or after inoculation, no mice at all died through the 40th day. Subinoculation proved that the surviving mice were all carriers of *Toxoplasma* organisms which could be recovered on the first passage. Surviving mice were also shown to be immune to reinoculation with the same strain of *Toxoplasma*.

[1] The antibiotics used were generously supplied as follows: Aureomycin, Dr. J. M. Ruegsseggar of Lederle Laboratories; Chloromycetin, Dr. Donald F. Walker of Parke Davis & Co.; Terramycin, Dr. Elliott R. Weyer of Chas. Pfizer & Co.; and Fumagillin, Dr. John S. Evans of the Upjohn Co. and Dr. George R. Hazel of Abbott Laboratories.

Some other observers have reported negative results with aureomycin, and it is very likely, judging from the lack of consistency of results, that there are strain differences in reaction to this drug.

Westphal (1950) reported that aureomycin administered to persons with positive dye tests for toxoplasmosis caused the titer to drop; however, Frenkel and Friedlander (1952) could not confirm this finding. Aureomycin apparently failed to check the *Toxoplasma* infection in two fatal adult cases of toxoplasmosis (Sexton *et al.*, 1952 and Kass *et al.*, 1952).

We have found no reports on the effect of terramycin on *Toxoplasma* infections in mice; however, Giroud and Gaillard (1951) used this drug (100 mg/kg. per day) in subcutaneously inoculated rabbits and found that it prevented systemic infection and blocked the development of cutaneous nodules, being superior to aureomycin in this respect.

Chloramphenicol was given to mice by Cross and Joseph (1949) in a diet suspension of 0.15, 0.2 and 0.3 per cent. The mouse infection was produced by the subcutaneous inoculation of 0.4 cc. of an emulsion in Tyrode's solution of infected mouse brain. Some prolongation of life was produced but all animals had died by the 11th day of the infection.

No report on fumagillin against toxoplasmosis has appeared, and no other laboratory has reported results on the other drugs in which comparison was made under strictly uniform conditions.

A number of investigators have reported negative results against toxoplasmosis with penicillin and streptomycin. Our experience with the first of these was similarly negative and is not reported here. We have not tried streptomycin but we are including here negative reports on neomycin, viomycin, prodigiosin, fracidin and R-2007[2].

METHODS

In all experiments a strain of *Toxoplasma gondii* isolated from a wild Norway rat was used (Eyles, 1952). The test animals were young mice (weight about 20 grams) of the NIH general purpose strain. In these mice the infection runs an invariably fatal course, the time to death varying with the size of the inoculum. In the experiments reported here intraperitoneal inoculations of 20,000 *Toxoplasma*, counted by means of a hemacytometer, were used. The parasites were obtained from the peritoneal exudate of infected mice and the number adjusted by diluting with physiological saline solution. In order to produce a uniform inoculum, exudates from five mice were pooled for each experiment. It has been determined experimentally that this inoculum is the equivalent of about 1000 median lethal doses of *Toxoplasma* organisms.

Treatment with the drugs began just after inoculation and continued for 14 days if the mice lived. The untreated controls were usually dead by 7 $\pm$ 1 days after inoculation. Clean animals maintained alongside the experimental animals showed no significant spontaneous mortality. Most of the drugs were admin-

[2] Viomycin was supplied by Chas. Pfizer and Company. The other drugs were supplied by Commercial Solvents Corporation.

istered in the diet to which the mice had free access. By using containers designed to minimize food spilling, it was found that mice usually took about four grams of diet per day, so drug dosages on a per kilogram basis were calculated using this figure.

In the case of the principal drugs studied, a range of dosages of the drugs approaching toxic levels was used. Twelve mice were given each dosage. Mice dying before the sixth day after inoculation were not considered as having died of toxoplasmosis and were not included in the tabulations.

For the drugs given only a screening-type test, a single dosage approaching the maximum tolerated dose was given to six animals, and the results were assessed by comparing the mean life span of the treated group with that of the controls.

FINDINGS

Comparative tests with broad spectrum antibiotics: Table 1 summarizes an experiment utilizing varying dosages of aureomycin, terramycin, fumagillin, and chloramphenicol. It can be seen that all four of these drugs produced prolongation of life in treated animals, at least at the highest dosages given, but they varied markedly in their effectiveness. The dosages of the different antibiotics are roughly comparable in that in each case the highest dosage was selected to approach the toxic level.

Aureomycin was by far the most effective of the drugs tested. A dosage between 100 and 200 mg. per cent in the diet resulted in 50 per cent survival for 10 days. Even with 400 mg. per cent only 25 per cent of the animals survived for 42 days; however, these animals were apparently cured as no infections developed when the tissues of the survivors were put into clean mice (two passages).

None of the other antibiotics prolonged life as long as 42 days at the dosages administered. A dosage of between 500 and 1000 mg. per cent terramycin did produce 50 per cent survival for 10 days but even when 2000 mg. per cent of the drug was given all mice died by the 22nd day.

With fumagillin fewer mice survived 10 days at 200 mg. per cent than at 100 mg. per cent, perhaps due to toxicity of the drug. When 100 mg. per cent in the diet was given, only 33 per cent survived 10 days and none lived longer than 12 days. Most of the mice dying showed *Toxoplasma* in the peritoneal exudate but a few did not have the parasites in demonstrable numbers.

Chloramphenicol appeared to be the least effective of the group. Administered in the diet at 2000 mg. per cent, 25 per cent lived 10 days and one mouse survived 23 days. At 1000 mg. per cent there was a slight but statistically significant prolongation of life, whereas when 500 mg. per cent was given there was no effect. All animals given chloramphenicol showed *Toxoplasma* organisms in the exudate when autopsied following death.

Since all of the drugs were being administered in massive dosages, only the results with aureomycin appear to be sufficiently significant to call for further study. Tissues from the four mice surviving 42 days in the present experiments were subinoculated into clean mice (two passages) and no infection developed.

This indicates that under some conditions cure can be effected with this agent. Experiments are planned to explore its action further.

TABLE 1

Effect of antibiotics on Toxoplasma *infections in mice induced by the intraperitoneal inoculation of 20,000 organisms. Drug administered for 14 days starting just after inoculation*

DOSAGE, (MG. PER CENT IN DIET)	NUMBER OF MICE	DAY OF DEATH*	PER CENT SURVIVING 10 DAYS	PER CENT SURVIVING 42 DAYS
Aureomycin				
400	12	*9, 10, 10,* 12, 12, 13, *18, 25, 32*	75	25
200	12	*8, 9, 9,* 11, *12,* 19, *21, 22, 24, 24,* 30	75	8
100	11	*7, 8, 8, 9, 9, 9, 10, 11,* 12, *21, 21*	37	0
Terramycin				
2000	11	*9,* 9, *10,* 14, 14, *16,* 19, 19, *21,* 21, *22*	73	0
1000	12	8, *9, 9, 10, 11,* 11, 12, *12, 13,* 17, 17, *18*	67	0
500	12	*7, 8, 8, 9, 9, 9,* 9, *10, 10, 11, 12*	17	0
Fumagillin				
200	12	*7, 7, 9, 9, 9,* 9, 9, *10, 10,* 10, 11, 11,	17	0
100	12	*7, 8, 8,* 8, 9, 9, *10, 10, 11, 11,* 11, *12,*	33	0
50	12	*7, 7, 8,* 8, 8, *9, 9, 10, 10,* 10, 10, 11	8	0
Chloramphenicol				
2000	12	*7, 7, 7, 8, 8, 8, 9, 9, 9, 11, 13, 23*	25	0
1000	12	*7, 7, 7, 7, 7, 8, 8, 8, 8, 9, 9, 10*	0	0
500	12	*7, 7, 7, 7, 7, 7, 7, 7, 8, 8, 8, 8,*	0	0
Controls—no treatment	8	*7, 7, 8, 8, 8, 8, 8, 9* (mean days to death —7.9)	0	0

* Italicized numeral indicates that *Toxoplasma* were found on autopsy.

TABLE 2

Negative trials of antibiotics against toxoplasmosis in mice

DRUG	DOSAGE	NO. OF MICE	MEAN DAYS TO DEATH—CONTROLS	MEAN DAYS TO DEATH—TEST ANIMALS	DELAY OF DEATH DUE TO DRUG
Viomycin	12 mg. once daily s.c.	6	7.33 ± 0.21	6.66 ± 0.34	−0.77 days
Neomycin	800 U. once daily s.c.	6	6.75 ± 0.16	7.17 ± 0.17	+0.42 days
	50–400 U. once daily s.c.	35	6.75 ± 0.16	7.03 ± 0.03	+0.28 days
Neomycin	1200 U. per mouse/day in diet	6	8.00 ± 0.00	7.33 ± 0.21	−0.67 days
Fracidin	0.025% in diet	6	6.90 ± 0.26	5.3 ± 0.34	−0.60 days
Prodigiosin	0.015% in diet	6	6.90 ± 0.26	6.7 ± 0.21	−0.20 days
R-2007	0.5 mg. once daily, s.c.	6	6.90 ± 0.26	6.00 ± 0.37	−0.90 days

Results of screening tests on other antibiotics: As can be seen from Table 2, none of the antibiotics listed had any effect on the *Toxoplasma* infection. The possibility remains that some effect might conceivably be demonstrated if the drugs were

differently administered; however, the negative results presented should be of aid as a guide in planning further experiments.

DISCUSSION

Terramycin had not been tested previously against mouse toxoplasmosis; however, the demonstrated effect confirms the observation of Giroud and Gaillard (1951) that this drug is effective against the organism in the rabbit. There is little likelihood that terramycin will be a drug of practical importance against the parasite as our dose of 2000 mg. per cent in the diet, which is the equivalent of about 4 grams per kg. per day, did no more than prolong life for a few days. This dosage is massive and much higher than the dosage attainable in man.

Fumagillin had not previously been tested against toxoplasmosis. Like terramycin, its activity is of theoretical rather than practical interest, for at dosages approaching toxicity only a slight prolongation of life resulted.

Chloramphenicol likewise only prolonged life at massive dosages. Our results confirm the observations of Cross and Joseph (1949) but do not indicate that the drug is important.

Further study is necessary before drawing the same conclusion with regard to aureomycin; however, the failure of the usual dosages of aureomycin to check the infection in two fatal cases of adult toxoplasmosis would indicate that this drug is of no practical importance. Against this we have the unconfirmed report of Westphal that aureomycin affects the serological titer as measured by the dye test, and the fact that in our experiments as well as those of some other observers the death of some mice was prevented.

A comparison of our study with the others in the literature does indicate strain differences in response to aureomycin. In the experiments of Steen (1950), the mice all lived when treated with aureomycin beginning about the time of inoculation, but these mice proved to be carriers of organisms which grew out in the first passage when tissues from the mice were put into clean animals. In our experiments some of the animals lived, but with our strain the four survivors did not produce infections in clean mice even though passed twice. In Summers' (1949) experiments mice lived only for a limited time even when the inoculum was much smaller than that used by us or by Steen. We hope to use strains of different response side by side in experiments at this laboratory in order to attempt to confirm the existence of these differences and to see if they parallel similar suspected differences in response to sulfonamides.

We have been struck by the parallel activity of the antibiotics against toxoplasmosis and against malaria. According to Coatney and Greenberg (1952) aureomycin, terramycin, chloramphenicol, and fumagillin are also active against *Plasmodium;* whereas the drugs we found inactive are for the most part, at least, inactive against malaria. Similarly, the drugs we found effective against toxoplasmosis are also effective against *Endamoeba histolytica.* It might be added that a similar parallel of antitoxoplasmic and antimalarial activity was found in other drug groups of diverse chemical nature (unpublished observations; see also Eyles and Coleman, 1952).

The experiments indicate that antibiotic activity against toxoplasmosis is real and is not uncommon. Perhaps there is hope that an antibiotic of practical effectiveness may appear in the future.

SUMMARY

The experiments described demonstrated statistically significant activity against mouse toxoplasmosis for aureomycin, terramycin, fumagillin, and chloramphenicol; however, with the possible exception of aureomycin, none is believed to be sufficiently active to be of practical importance. Aureomycin produced apparent cures in some mice, but only at extremely high dosages, higher than those attainable in man. Comparison of the results of these experiments with those of others indicates the existence of strains of *Toxoplasma* differing in their response to this drug.

Viomycin, Neomycin, Fracidin, Prodigiosin and R-2007 produced no effect on mouse toxoplasmosis under the conditions of our experiments.

LITERATURE CITED

COATNEY, G. R., AND GREENBERG, J. 1952, The use of antibiotics in the treatment of malaria. In preparation for publication.

CROSS, J. B., AND JOSEPH, H. 1949, Chloromycetin and experimental toxoplasmosis, *Texas Rep. Biol. & Med.* **7:** 406.

EYLES, D. E. 1952, *Toxoplasma* in the Norway Rat, *J. Parasitol.* **38:** 226–229.

EYLES, D. E., AND COLEMAN, N. 1952, Tests of 2:4-diaminopyrimidines on toxoplasmosis, *Pub. Health Rep.* **67:** 249–252.

FRENKEL, J. K., AND FRIEDLANDER, S. 1952, Toxoplasmosis. Pathology of neonatal disease. Pathogenesis, diagnosis, and treatment. Pub. Health Bull. No. 141, Government Printing Office, Washington, D. C.

GIROUD, P., AND GAILLARD, J. A. 1951, Action comparée de la Terramycine et de l'Aureomycine sur les Toxoplasmoses, *Compt. rend. Acad. de Sc.* **232:** 1457–1459.

KASS, E. H., ANDRUS, S. B., ADAMS, R. D., TURNER, F. C., AND FELDMAN, H. A. 1952, Toxoplasmosis in the human adult, *Arch. Int. Med.* **89:** 759–782.

SEXTON, R. C., EYLES, D. E., AND DILLMAN, R. E. 1952, Adult toxoplasmosis, *Am. J. Med.*, in press.

STEEN, E. 1950, Acute experimental toxoplasmosis treated with Aureomycin, *Acta path. et microbiol. Scandinav.* **27:** 844–850.

SUMMERS, W. A. 1949, The effects of oral administration of aureomycin, sulfathiazole, sulfamerazine and 4,4′-diaminodiphenylsulfone on toxoplasmosis in mice, *Am. J. Trop. Med.* **29:** 889–894.

THALHAMMER, O., SCHNABL, E., AND MERITSCH, H. 1951, Behandlungsversuch exp. Toxoplasmose mit Sulfonamid und Aureomycin, *Weiner Ztschr. f. Med.* **32:** 262–266.

WESTPHAL, A. 1950, Das vorkommen von Toxoplasmose in Deutschland und ihre Behandlungamegliekeit mit Aureomycin, *Z. Tropenmed. u. Parasit.*, **1:** 526–533.

RESPONSE OF THE SYRIAN HAMSTER TO TWO STRAINS OF RABIES STREET VIRUS BY RECTAL INSTILLATION

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The rabies virus, strain V308, used in the present study was isolated in Swiss albino mice from a dog brain sent to this laboratory for a routine examination for rabies. The history of this strain was given in a previous paper which dealt with electron microscopy studies of Negri bodies (Reagan *et al.*, 1950). This strain was pathogenic for two species of bats (Reagan *et al.*, 1951) and for the Syrian hamster (Reagan *et al.*, 1951). Six 4-week-old Swiss albino mice were inoculated intracerebrally with 0.03 ml. of a 10 per cent virus-bearing brain suspension of the second mouse passage which titrated $10^{-4.0}$ in mice. Six days after injection the mice showed tremors and paralysis. The brains of 4 paralyzed mice were removed aseptically. Touch preparations from the Ammon's horn of these brains were stained with Sellers stain (Sellers, 1927), and upon examination by optical microscope were found to contain numerous Negri bodies. A 10 per cent physiological saline suspension of these virus-bearing brains was used to initiate a part of the present study. The titer of this material intracerebrally in mice was $10^{-3.5}$.

The second strain of rabies virus, V644, used in this study was also isolated in Swiss albino mice from a dog brain sent to this laboratory. This strain was inoculated into mice, harvested, prepared, and examined for Negri bodies in the same manner as the above-mentioned strain. The virus-bearing mouse brains infected with this strain (V644) contained numerous Negri bodies and titrated $10^{-3.0}$ intracerebrally in mice.

The titer of the two strains was exceedingly low due to the long storage of the strains in the −40°C. deep freeze for several months before initiation of the experiment. The two strains were confirmed to be rabies virus strains by neutralization tests conducted in Swiss albino mice. The rabies virus immune serum used in the neutralization tests was furnished by the Lederle Laboratories.

EXPERIMENTAL METHODS

Two series of studies were undertaken, Series A and Series B. Twelve healthy hamsters (age, 17 days) were divided into two groups. Six hamsters were inoculated rectally with 0.1 ml. of the virus-bearing brain suspension of strain V308 (series A) and 6 hamsters were inoculated in the same manner with virus-bearing brain material of strain V644 (series B). A 19 gauge hypodermic needle, the point of which had been cut off to make it blunt and smoothed by filing was used for injection. The needle was coated with vaseline and inserted half an inch into the lumen of the rectum. After rabid symptoms appeared the brains of ham-

TABLE 1

Response of hamsters to rabies virus by rectal instillation

PASSAGE	STRAIN	NO. OF ANIMALS* SHOWING SYMPTOMS	MIN.-MAX.† INCUBATION PERIOD IN DAYS
1	V308	6/6	8–10
2			8–11
3			8–10
4			8–10
5			7–8
1	V644	6/6	6–8
2			6–8
3			6–8
4			6–8
5			7–8

* Numerator denotes no. of animals succumbing to the virus, and denominator denotes no. exposed.

† Incubation period = time between exposure to virus and onset of symptoms.

TABLE 2

Response of hamsters inoculated with virus-bearing suspensions of organs from the fifth rectal passage

ORGANS	STRAIN	INTRACEREBRAL INOCULATION		RECTAL INOCULATION	
		No. of* hamsters showing symptoms	Min.-max.† incubation period in days	No. of* hamsters showing symptoms	Min.-max.† incubation period in days
Heart	V308	3/3	5–7	3/3	5–7
Liver			6–8		6–8
Lungs			6–8		6–8
Kidneys			6–8		6–8
Intestines		0/3	—	0/3	—
Brain		3/3	5–7	3/3	5–7
Spinal cord			5–7		5–7
Spleen			6–8		6–8
Heart	V644		5–7		5–7
Liver			6–8		6–8
Lungs			6–8		6–8
Kidneys			6–8		6–8
Intestines		0/3	—	0/3	—
Brain		3/3	5–7	3/3	5–7
Spinal cord			5–7		5–7
Spleen			6–8		6–8

* Numerator denotes no. of animals succumbing to the virus, and denominator denotes no. exposed.

† Incubation period = time between exposure to virus and onset of symptoms.

sters from both series were removed aseptically and examined for the presence of Negri bodies. Numerous Negri bodies were found in both A and B series. The brains of hamsters from each series were pooled separately, ground with alundum and diluted to a 10 per cent suspension with physiological saline. Virus-bearing brain material from series A and B were serially passaged for 5 passages by rectal instillation, as shown in Table 1. Hamsters showing rabid symptoms after the 5th serial passage had the following organs aseptically removed: heart, liver, lungs, spleen, kidneys, brain, spinal cord, and pooled intestines and feces. Like organs from each series were pooled, ground with alundum and diluted to a 10 per cent suspension with physiological saline. Salivary glands were not removed from these hamsters due to the difficulty of dissecting these organs. Intestines and feces, and lungs were treated with diethyl ether in order to elimiinate bacterial contamination. Forty-eight hamsters were divided in 16 groups of 3 hamsters each and each group was inoculated intracerebrally with one of the above suspensions. The inoculum in all cases was 0.06 ml. per hamster. Simultaneously 0.2 ml. of the same suspensions were instilled rectally into the same number of hamsters.

RESULTS AND DISCUSSION

The results in Table 1 indicate that all hamsters showed symptoms of rabies when inoculated rectally with strains V308 and V644. From the nature of the results recorded in Table 2 it is evident that all organs except the intestines and feces contained sufficient rabies virus to produce symptoms of rabies regardless of the route of inoculation. The identity of the virus was confirmed by the presence of numerous Negri bodies in stained touch preparations from the Ammon's horn of brains removed from animals showing rabid symptoms.

In a previous paper (Reagan *et al.*, 1951) hamsters infected intracerebrally and intranasally with strain V308 showed the furious form of rabies with such manifestations as extreme irritability, spasms, excessive salivation, inability to swallow, and occasional guttural cries. However, hamsters infected rectally in this study with strain V308 and V644 showed no furious form of rabies. The symptoms in these animals were manifested by excessive scratching of the rectal area, with some salivation, followed by paralysis and prostration. It was significant to note that hamsters inoculated intracerebrally with infected organs from the fifth rectal subculture (strain V308 and V644) did not display a furious form of rabies.

SUMMARY

Two strains of rabies virus isolated from dog brains, one (V308) passed three times in Swiss albino mice and the other (V644) passed twice in Swiss albino mice, have been successfully transmitted rectally for 5 serial passages in the Syrian hamster. Organs (heart, liver, lungs, spleen, kidneys, intestines and feces, brain, spinal cord) from the fifth serial passage of each strain were inoculated rectally and intracerebrally into hamsters. Regardless of the route of inoculation all organs tested were infective except the pooled feces and intestines.

These two strains of rabies street virus produced paralytic rabies in hamsters when the virus was administered rectally.

REFERENCES

REAGAN, R. L., AND BRUECKNER, A. L. 1950, Electron micrographs of Negri bodies found in rabies, *J. Infect. Dis.* **87:** 213–216.

REAGAN, R. L., AND BRUECKNER, A. L. 1951, Transmission of a strain of rabies virus to the large brown bat (Eptesicus fuscus) and to the cave bat (Myotis lucifugus), *Cornell Vet., XLI*: 295–298.

REAGAN, R. L., H. ODETTE LINEWEAVER, DOROTHY SCHENCK, AND BRUECKNER, A. L. 1951, Studies of rabies street virus in the Syrian hamster, *J. Bact.* **62:** 391–393.

SELLERS, T. F. 1927, A new method for staining Negri bodies of rabies, *Am. J. Pub. Health* **17:** 1080–1081.

YAWS ON GUAM: TREATMENT AND ENVIRONMENTAL CONSIDERATIONS

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Yaws has been noted frequently in reports from Guam for the last fifty years. Before 1942 attention was given principally to sufferers within easy reach of the hospital, and those particularly with lesions involving the nasopharynx (gangosa). Insanitary conditions on a magnified scale during the recent occupation of the island increased the spread of the infection. From December 1944 through the calendar year 1945, treatment was given to 2,246 persons; in 1946 to 1,322; and in 1947 to an additional 385. Only 19 cases were reported in 1948. Many adults were treated for their first infection and older children for relapses or reinfections associated with rapid spread among younger children. During the period from July 1945 through April 1946, 2,521 persons each received 0.04 gm. of mapharsen per week for 8 weeks.

In February 1949, children with infectious sores of yaws were found being treated for contagious impetigo. One physician was beginning to doubt the efficacy of his sulfathiazol topical application. Schools in the centers of population were visited and careful examinations revealed a substantial number of cases of active yaws. The search was extended well beyond the general range of treatment given in 1945 and 1946. Four outermost villages, least accessible by road, were visited, all the schoolchildren examined and diagnostic sessions held in each dispensary for those of preschool ages. For purposes of later comparison, it should be stated that the nearer villages, numbered 1 and 3, have 353 houses and a combined population of 2,401. Villages 2 and 4 have 279 houses and 1,578 people. Villages 2, 3 and 4 gave a complete response, every child on their registers being examined.

In the first school examined, that at village No. 2, the predominant relapse character of the lesions was easily apparent. The relapse ulcerations occurred with a violence not observed in Central and South America. The change from unbroken skin to serum-filled ulcers adjacent to old or fresh scars in younger children came with little or no proliferative phase. In addition, solitary granulomas were noted in older children whose scars and history would presuppose a stabilized infection and some degree of immunity after three to five years. These granulomas were common over the bony prominences of ankles, shins, knees, hips, elbows and shoulders. The granuloma quickly replaced the excoriation or abrasion of trauma and was more frequent in boys. In many instances such granulomas were followed within a short period by relapse ulcers elsewhere on the body.

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Another feature not previously observed was the excessive number of flies present in and about the schoolhouse. As pupils joined the examination line more flies were noted. Most were feeding avidly on the serum-filled ulcers and were so persistent that they were driven off with difficulty. The vicinity of the school was observed to be relatively free of flies before the opening of the daily session. Observers stationed at various points toward the edge of the village reported flies riding in, clustered on the sores, in the morning and equal numbers on children returning home in the evening. This experience was repeated in the other three villages. It was accentuated when the preschool age children with their greater frequency of open sores came to the diagnostic sessions in the dispensaries. The flies most commonly associated with sores were of small size with a bluish hue. Identification was not made as an entomologist was not available. Some so-called "large bluebottle" flies were present also but not in great numbers and rarely found on or near sores.

To reduce the fly population, a 7.5 per cent solution of DDT in clear kerosene was sprayed from shoulder-sling tanks on interiors of houses beginning one week before the first treatment clinic and for each of the three weeks following. The same spray was used in and about the Quonset dispensary and schoolhouse early on the morning of each treatment clinic. A 5 per cent DDT in diesel oil was applied by high-pressure cone-spray under and about each house on the days of the interior spraying. Both types of spraying were done thereafter on four monthly occasions until interrupted by a typhoon. Householders were instructed to clean up their properties and to place all trash at the edge of streets and roadways for the daily collection by the village public works truck.

Treatment of the patient comprised 300,000 units of procaine penicillin in 2 per cent aluminum monostearate, intragluteally, at three to four-day intervals for a total of 1.2 million units; bismuth subsalicylate 0.2 gm. was given in the other buttock at the time of each penicillin dose. Rapid epithelization occurred in the sores, but late type granulomas melted slowly. There were 34 such cases that remained unhealed after the fourth treatment and were given three to six additional bismuth injections at weekly intervals by the dispensary nurses. Thirty-three treatment sessions were missed by 24 patients. Half-doses of penicillin and bismuth were given to 67 children less than 24 months of age.

A total of 1,945 children were examined: 803 being diagnosed as having active disease, 702 judged inactive and 440 without yaws. It was necessary to include in Table 1 those children seen for the first time when follow-up examinations were being made. This accounts for 35 of the 38 untreated in the active group and for 109 (24.9 per cent) of the 437 untreated in the inactive group. The age at onset was determined chiefly from those having their first crop of ulcers or having their first relapse within the first 12 months of clinical disease. The median age at onset was 7 years; that for active yaws 6 years and for inactive disease 10 years.

Follow-up observation of treated and untreated cases was made five to eight months later and for a special 58 cases with other conditions, a further re-examination was made at nine months. Failure to appear for these examinations

accounted for 15.5 per cent of the treated and 14.3 per cent of the untreated cases. For villages No. 3 and No. 4, a deduction of 10 and 4 should be made from the infectious relapse total of 87, as these had no disease on first examination but showed yaws on follow-up; five of these experienced a relapse in the 5 to 8 month period. Thus, infectious relapse occurred in 9.5 per cent of 772 treated patients, seven of whom were inactive initially.

The late relapse type includes 32 untreated inactives and 9 without evidence of yaws on first examination. Treated active infections contributed 197 and

TABLE 1

Yaws on Guam

Distribution by age, sex, onset, stage of disease and type of relapse

AGE	ONSET			ACTIVE			INACTIVE			INFECTIOUS			LATE		
	Boy	Girl	Both	Boy	Girl	Both	Boy	Girl	Both	Boy	Girl	Both	Boy	Girl	Both
<1	9	20	29	9	15	24	3	4	7	1	3	4	1	2	3
1	45	30	75	22	22	44	11	9	20	8	6	14	4	5	9
2	53	41	94	44	48	92	37	32	69	13	6	19	10	8	18
3	31	29	60	48	34	82	30	25	55	6	7	13	9	10	19
4	29	22	51	40	39	79	23	25	48	1	5	6	7	6	13
5	18	28	46	32	22	54	23	24	47	4	1	5	11	2	13
6	19	18	37	22	21	43	19	24	43	2	2	4	11	15	26
7	7	9	16	30	24	54	22	13	35	2	2	4	19	4	23
8	9	14	23	28	26	54	25	18	43	3	5	8	16	12	28
9	13	11	24	27	30	57	29	32	61	0	1	1	17	14	31
10	4	9	13	22	25	47	18	14	32	2	3	5	10	12	22
11	7	10	17	19	18	37	14	16	30	2	0	2	5	8	13
12	5	3	8	20	26	46	18	15	33	0	1	1	15	7	22
13	7	4	11	13	10	23	15	17	32	0	0	0	4	4	8
14	2	1	3	10	15	25	16	15	31	1	0	1	6	6	12
15	2	1	3	7	10	17	20	22	42				6	7	13
16	1	2	3	8	5	13	13	17	30				2	2	4
17	2	0	2	2	4	6	12	11	23				1	0	1
18	0	2	2	1	2	3	3	9	12				0	0	0
19	0	0	0	1	1	2	1	4	5				1	0	1
20	0	1	1	0	1	1	1	3	4						
Total	263	255	518	405	398	803	353	349	702	45	42	87	155	124	279

treated inactive infections 46 to the total of 23.1 per cent late relapse. In the course of follow-up, 55 preschool children were seen for the first time in village No. 1, 15 with active, 15 with inactive infections; but the register showed 185 still unexamined. In village No. 2, 156 holdouts were examined in follow-up, with 29 active and 83 inactive cases, to complete the register. Practically all relapses occurred at four to five months post-treatment; subsequent examinations and village nurse reports disclosed none after the seventh month.

There remained the question of the effect of fly control on relapses and new yaws infections. Nine months after the treatment sessions ended, an island-wide survey for mosquito larvae was made. Fly population estimates were found to

follow in close parallel with mosquito findings. In village No. 1 where all the active cases and half of the inactive yaws had been treated but where sanitary cooperation was next to the poorest, the relapse rate was next to the highest. However, this situation is complicated by the great reservoir of untreated infections comprising the preschool children not examined at the first diagnostic sessions, a follow-up failure almost twice that of the other three villages combined, and those not examined at any time. In village No. 2 all active infections were treated but only six of 214 inactives were treated; 29 active and 83 inactive were seen in follow-up as an untouched reservoir, yet the total relapse rate was only 4.7 per cent. Practically no flies were found in this village and sanitary cooperation had been excellent. In village No. 3 all on the register were examined at the outset and all active and half the inactive cases were treated, yet it had a total relapse rate of 36.1 per cent. In addition, 16 new infections appeared in the seven months following initial examination. Sanitary effort was the weakest and fly population the greatest in this village. Village No. 4 was similar to Nos. 1 and 3 in that all its children were examined initially; treatment was given to all its active and half its inactive cases; it was second best for sanitary effort and had a low fly population; the total relapse rate was 9.3 per cent.

Other conditions in the environment were investigated. Scabies was found in 13 children, 10 of them in villages No. 1 and No. 3, ranging in age from 4 to 15 years. Benzyl benzoate was used for all, beginning about two months after the treatment sessions. One case without yaws was cleared in the same time as the 10 others. Two had an infectious relapse and 3 had a late type relapse, but it is believed that, since 11 were originally diagnosed as having acute yaws, the scabies infestation and its treatment did not adversely affect the course of the yaws.

A flurry of chickenpox struck villages 1 and 2, with 30 and 6 cases respectively, about three months after the yaws treatment. All had been treated for acute yaws and no chickenpox occurred in children found to be free of yaws. Infectious relapse was seen in 68 per cent of the cases, accounting for the entire 6 relapses of this type in village Number 2. It was noted that the speed and severity of relapse appeared to be influenced by the complicating chickenpox, even though all had healed promptly following treatment.

Ants were implicated by a few relapsing cases when seen at the first diagnostic sessions. On follow-up examinations, 9 relapses were seen associated with ant bites. Two were of the infectious type, seven of the late type; the two former, and three of the latter occurred in village No. 3 with the poorest sanitation. Scratch excoriations, even more than in scabies, appeared to be the starting point.

SUMMARY

A violent type of yaws relapse was found in 4 outermost villages of Guam; 1,030 children were treated with 4 doses each of depository penicillin and bismuth subsalicylate.

The lowest relapse rate five to eight months after treatment was found in the village having the best DDT control of its flies and the best trash clean-up; the worst rate occurred in the village having poorest success with its fly population. A third village closely approximated the first one in accomplishment but the fourth village had too many cases left untreated to permit valid interpretations.

Scabies and its treatment did not appear to influence yaws relapse but chickenpox could be considered an exciting factor to a high degree.

INTRADERMAL REACTIONS TO SCHISTOSOME AND FILARIA ANTIGENS IN PATIENTS BEFORE AND AFTER TREATMENT OF THE PARASITIC INFECTIONS

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Intradermal reactions are valuable in the diagnosis of schistosomiasis and filariasis. The test antigens for the diagnosis of schistosomiasis are prepared chiefly from extracts of the cercariae or adult forms. The dog and rat filarial worms, *Dirofilaria immitis* and *Litomosoides carinii* respectively, are used as sources for skin testing antigens in filariasis.

Numerous reports have been made on the interpretation and validity of the tests and extensive work has been performed on large numbers of patients (Culbertson, 1941). Various facts, however, still remain to be investigated. Of these, the duration of the reaction in treated and untreated patients is a point of major importance. Also of interest is the study of the differences in the skin reactions observed when test antigens are prepared from related parasites and from the specific infective organisms.

MATERIALS AND METHODS

A one per cent suspension of the well pulverized worm material was made in sterile 0.85 per cent physiologic salt solution, incubated at 37°C. for one hour with frequent shakings, and left at room temperature (28°C.) overnight. The suspension was then centrifuged for 15 minutes at 4,000 r.p.m. and the supernate removed with a pipette to be used as the stock 1:100 dilution of antigen. This was further diluted with physiologic saline to 1:10,000 and sterilized by immersion in a water bath at 56°C. for one hour on three consecutive days. The sterility of the antigen was tested on fluid thioglycollate medium.

For the skin test 0.1 cc. volume of the antigen and of physiologic salt solution (0.85%) as a control were injected intradermally with a No. 27 gauge needle in the forearm. The bleb which formed immediately after injection was outlined with ink. Positive reactions were those in which the bleb extended beyond the ink line as observed 10 to 15 minutes after the antigen was injected.

Patients with schistosomiasis

The individuals infected with schistosomiasis *mansoni*, as determined by the presence of ova in the stools or rectal mucosa, were all patients of the University Hospital, School of Medicine, School of Tropical Medicine. They came from endemic foci in Puerto Rico where they are constantly exposed to infection. Some were hospitalized for varying periods of time and others attended the out patient clinic.

The drug utilized for treatment was mainly Fuadin, given intramuscularly. Although the schedule of treatment varied, all patients received at least two series of injections, each series consisting of a total of 60 cc. of the drug. Most of

the patients, however, received additional series of injections, depending on drug tolerance and on the results of the fecal and rectal biopsy examinations. Ten patients were treated with urea stibamine (Merck), no other drug being administered.

The stools and rectal biopsies were examined for ova of *S. mansoni* at frequent intervals before and after treatment. These examinations were performed for prolonged periods of time and until no ova were found in the stools or rectal mucosa. The patients with no symptoms and with stools and biopsies negative for ova were arbitrarily considered as cured.

All patients with schistosomiasis were tested with a 1:10,000 dilution of antigen prepared from:

a) cercariae of *Schistosoma mansoni*
b) the liver fluke, *Fasciola hepatica*, and
c) the frog fluke, *Pneumoneces medioplexus*.

The last two parasites have been shown to possess an antigenic substance apparently common to all trematodes which reacts with other members of the group (Culbertson, Rose and Oliver-González, 1947). The patients were tested before treatment and as many times as was possible after treatment.

Patients harboring microfilariae of Wuchereria bancrofti

Individuals with circulating microfilariae, as determined by examinations of nocturnal blood, were patients of the University Hospital and inmates of the Insular Penitentiary[1] at Guaynabo, P. R. Some of the patients were treated with diethylcarbamazine (1-diethyl-carbamyl-4-methyl-piperazine dihydrogen citrate), a compound which causes rapid disappearance of the circulating microfilariae within 48 hours of its administration. A dose of 2 mgm. per kilo of body weight, three times daily for 10 days, was the regime usually followed (Santiago-Stevenson, Oliver-González, and Hewitt, 1947).

A second group of patients were treated with neostibosan and other antimonial compounds. During treatment with the antimonial compounds the microfilariae disappeared gradually, the blood becoming negative 6 to 12 months after treatment (Culbertson, Rose, Hernández-Morales, Oliver-González, and Pratt, 1947).

The patients were skin-tested before and after treatment. The test antigens used were 1:10,000 dilutions prepared from the adult dog and the rat filarial worm, *Dirofilaria immitis* and *Litomosoides carinii*. A 1:1,000 dilution of antigen prepared from the microfilariae of *W. bancrofti* was also used (Oliver-González and Bercovitz, 1944).

RESULTS

Skin reactions in schistosomiasis patients before and after treatment

Two hundred and forty-six patients were tested before treatment with the 1:10,000 dilution of cercarial antigen. Of these 235, or 95.5 per cent, gave posi-

[1] The author wishes to acknowledge the cooperation of Dr. E. Méndez Polo, physician in charge, and Mr. José M. Dobal, chief of the Penitentiary's hospital laboratory.

tive intradermal reactions. The percentage of positive reactions to the cercarial antigen one-half, one, two and four years after treatment did not vary significantly from the percentage of positive reactions (91.8, 97.2, 96.4 and 92.9, respectively, as compared with 95.5) before treatment (Table 1).

There was a marked difference, however, in the percentage of positive reactions to the antigens prepared from *Fasciola hepatica* and *Pneumoneces medioplexus*. In the case of the antigen from *Fasciola*, only 51.9 per cent gave positive reactions before treatment, and the percentage of positives decreased with

TABLE 1

Number of positive skin reactions to 1:10,000 dilution of trematode antigens in individuals infected with Schistosoma mansoni

ANTIGEN	BEFORE TREATMENT SCHISTOSOME OVA IN STOOLS OR RECTAL MUCOSA		TIME AFTER TREATMENT—YEARS; NO OVA IN STOOLS OR RECTAL MUCOSA							
			0.5		1		2		4	
	No. of patients tested	No. of positive reactions -%	No. of patients tested	No. of positive reactions -%	No. of patients tested	No. of positive reactions -%	No. of patients tested	No. of positive reactions -%	No. of patients tested	No. of positive reactions -%
Cercariae of *S. mansoni*	246	235-95.5	208	191-91.8	180	175-97.	166	160-96.4	140	130-92.9
Adult Fasciola hepatica	231	120-51.9	200	89-44.5	174	41-23.5	150	20-13.3	94	10-10.6
Adult *Pneumoneces medioplexus*	210	197-93.8	176	150-85.2	101	54-53.4	100	38-38.0	90	26-28.9

TABLE 2

Number of positive skin reactions to antigens from filarial worms in patients, before and after treatment with Hetrazan and various antimonial compounds

ANTIGEN AND DILUTION	BEFORE TREATMENT MICROFILARIAE IN BLOOD		TIME AFTER TREATMENT (YEARS); NO MICROFILARIAE IN BLOOD					
			0.5		1		2	
	Number of patients tested	Number of positives %	Number of patients tested	Number of positives %	Number of patients tested	Number of positives %	Number of patients tested	Number of positives %
1:10,000 adult *Dirofilaria immitis*	68	67-98.5	60	57-95.0	44	43-97.8	39	36-92.3
1:10,000 adult *Litomosoides carinii*	68	65-95.6	60	60-100.0	44	40-90.9	39	37-94.9
1:1,000 microfilariae of *Wuchereria bancrofti*	68	67-98.5	60	54-90.0	44	39-88.4	39	34-87.2

time. Thus, from 51.9 before treatment the percentage of positives came down to 10.6 four years after treatment. Although the per cent of positives to the antigen from *Pneumoneces* before treatment compares with that to the cercarial antigen (93.8 vs. 95.5), this decreased to 28.9 in the fourth year after treatment.

Skin reactions in patients with blood microfilariae

The percentage of positive reactions to the three types of filarial antigens was more or less the same before as after treatment. This was so even as late as two years after the end of medication (Table 2).

DISCUSSION

Positive reactions to antigens from cercariae of *S. mansoni* were still observed in 95.5 per cent of patients infected with *S. mansoni* who received adequate amounts of drug even as late as four years after treatment. Although these individuals might have become reinfected, the chances are that they did not, since no ova reappeared in the stools or rectal mucosa even after repeated examinations. It seems, therefore, that the test is not of prognostic value, since it was positive long after the patients were considered to be cured.

The cutaneous reaction seems to be influenced by the duration and the intensity of the infection. Katzen and Most (1946) reported that in the American soldiers who became infected with *Schistosoma japonicum* while in the Pacific areas the intradermal response diminished and became negative several months after treatment. The exposure of the soldiers to infection may have been for a limited period of time and in only a few instances, as compared to likelihood of repeated exposure of individuals residing all their lives in infested areas. The adequate treatment of recently acquired infection also lessens the possibility of involvement of the liver and other organs by the eggs of the parasite. In recently treated individuals who have undergone numerous reinfections, treatment does not affect the eggs of the parasite that lodge in the liver. These eggs serve as a constant source of antigen which keeps the host sensitized for a certain period after death of the adult worms. Furthermore, in heavily infected individuals, small numbers of adult worms may remain alive in spite of treatment and serve to immunize the host. In this case the number of live worms may be so small that the infection can not be detected by stool or biopsy examination. The differences between the length of time during which the patients reported upon by Katzen and Most (1946), and those from other endemic areas reacted intradermally to cercarial antigen, may therefore, be ascribed to total termination of infection and of parasitic involvement in the first case, and further antigenic stimulation by eggs and a small number of worms in the second.

The number of positive reactions to antigens from *Fasciola hepatica* and *Pneumoneces medioplexus* adults decreased markedly after treatment. This suggests that the antigen common to the three species of trematodes is associated with the adult rather than the larval forms. Antigenic differences between adult and larval stages have been demonstrated previously (Oliver-González, 1941). This would imply that when the cercarial antigen is used in the skin test, the positive reactions obtained are against common antigens present in the adult and the cercariae, as well as to specific antigens in both forms. There is also the possibility that the antigens from *Fasciola hepatica* and *Pneumoneces medioplexus* are less stable than the cercarial antigen. This may be so particularly, after storage at 6°C. for as long a period as four years, (Pratt, Oliver-González, 1947).

The per cent of positive skin reactions to the three types of filarial antigens remained approximately the same before and after treatment (Table 2). This is of great interest since it is unlike the results obtained with the trematode antigens as tested in the individuals infected with *S. mansoni*. First of all, cross reac-

tivity with other nematode infections such as ascaris and hookworm should be considered. Positive reactions to high dilutions of filarial antigens were obtained in about the same number of individuals, whether negative or positive for microfilariae (Oliver-González, and Hernández-Morales, 1945). There was a high incidence of intestinal parasitic infections among the microfilarial positives and negatives, suggesting, therefore, that the positive reactions in the non-filarial patients were probably due to cross reactions with the intestinal nematode. Athough the individuals tested were not examined for intestinal parasites, it is very likely that such infections were common among them. Also, since the adult and filarial embryos were killed as a result of treatment, it was not to be expected that specific filarial antibodies would be present as late as two years after treatment.

Positive reactions to filarial and schistosome cercarial antigens are, therefore, obtained in a high per cent of individuals even after they have been adequately treated for the specific infection. Although the test is of great aid in diagnosis, the results indicate that it can not be used as a guide to the efficacy of treatment. This is particularly so in chronic schistosomiasis in which conditions the liver and other organs are involved.

SUMMARY

Individuals infected with *Schistosoma mansoni* were skin-tested with extracts of (1) dry and powdered cercariae of *S. mansoni*; and (2) dry and powdered adult *Fasciola hepatica* and *Pneumoneces medioplexus*. The skin tests were performed before treatment and for prolonged periods of time up to four years after treatment of the parasitic infection.

Using the cercarial antigen, the reactions were positive in above 90 per cent of the patients in all instances both before and after treatment. With the antigens from *Fasciola hepatica* and *Pneumoneces medioplexus* the percentage of positive reactions diminished greatly after treatment. The schistosome ova in the liver and other organs may continue to sensitize the host for short periods of time after death of the adult worms as a result of treatment. Also, positive intradermal reactions may be due to infection with a small number of adult worms which can not be detected by fecal or biopsy examinations.

Individuals with circulating microfilariae of *Wuchereria bancrofti* were skin-tested with antigen from *Dirofilaria immitis* and *Litomosoides carinii* and with antigen prepared from concentrated microfilariae. Positive reactions to the three types of antigen were observed in over 90 per cent of the individuals tested before as well as two years after treatment.

REFERENCES

CULBERTSON, J. T. 1941, *Immunity against Animal Parasites*, Col. Univ. Press, 161–162, 210–211.

CULBERTSON, J. T., ROSE, HARRY M. AND OLIVER-GONZÁLEZ, J. 1947, Skin tests in schistosomiasis *mansoni* with antigen from heterologous worms (Pneumoneces, Planaria), *J. Infec. Dis.* **80:** 218–221.

CULBERTSON, J. T., ROSE, H. M., HERNÁNDEZ-MORALES, F., OLIVER-GONZÁLEZ, J. AND

PRATT, C. K. 1947, The experimental chemotherapy of filariasis *bancrofti*, *P. R. J. Pub. H. & Trop. Med.* **22:** 139–173.

KATZEN, B., AND MOST, H. 1946, Cercarial antigen skin test in diagnosis and management of schistosomiasis *japonica*, *Bull. U. S. Army Med. Dept.* **6:** 613–616.

OLIVER-GONZÁLEZ, J. 1941, Dual antibody basis of acquired immunity in trichinosis, *J. Infec. Dis.* **69:** 254–270.

OLIVER-GONZÁLEZ, J., AND BERCOVITZ, Z. T. 1944, Precipitin reactions with antigen prepared from microfilariae of *Wuchereria bancrofti* (Preliminary report), *Am. J. Trop. Med.* **24:** 315–316.

OLIVER-GONZÁLEZ, J. AND HERNÁNDEZ-MORALES, F. 1945, Common antigens among filaria and other nematode parasites of man, *J. Infec. Dis.* **77:** 92–95.

PRATT, C. K., AND OLIVER-GONZÁLEZ, J. 1947, Intradermal reactions to fresh and stored antigens prepared from cercariae of *Schistosoma mansoni*, *P. R. J. Pub. H. & Trop. Med.* **22:** 254–256.

SANTIAGO-STEVENSON, D., OLIVER-GONZÁLEZ, J., AND HEWITT, R. 1947, Treatment of filariasis *bancrofti* with 1-diethyl-carbamyl-4-methyl-piperazine hydrochloride (Hetrazan), *J. A. M. A.* **135:** 708–712.

IMMUNITY IN FILARIASIS OF EXPERIMENTAL ANIMALS

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This paper describes an investigation of immune bodies in the filarial infections of experimental animals. A summary of the ways in which immune responses may be detected, is given at the beginning of the discussion. For a number of reasons all the points in the summary were not investigated, and for simplicity the experimental work has been classified as:

A. Experiments *in vitro*
B. Experiments *in vivo*
C. Skin tests.

METHODS

The filarial infections which were available for study at the National Institute for Medical Research were *Dirofilaria repens* in dogs, a filarial worm (as yet undescribed) in monkeys, and *Litomosoides carinii* in cotton rats. For all practical purposes these infections are nonpathogenic; as is pointed out in the discussion, this nonpathogenicity probably had an important influence on the results obtained. Microfilariae of *D. repens* and of the monkey parasite developed satisfactorily to infective larvae in *Aedes aegypti* mosquitoes and migrated eventually into the proboscis, and microfilariae of *L. carinii* developed in the mite *Liponyssus bacoti*. Dried *D. immitis* worms were obtained from Sir. Neil H. Fairley for use as antigen.

As a first step in this investigation rabbits were immunized with filarial antigen so as to provide sera known to contain large amounts of antibodies. Six rabbits of approximately the same age and weight were given a course of subcutaneous injections of *D. immitis* antigen. The antigen was prepared by grinding the dried worms in a tissue grinder and making a 1/500 suspension in sterile Ringer's solution. They were not extracted lest any fraction of immunological importance be removed. Rabbits I and II received 1 mg./kg. body weight, rabbits III and IV received 10 mg./kg., and rabbits V and VI received 20 mg./kg. The first two injections were made at an interval of three weeks, and subsequent injections were given every ten days. The rabbits were bled at intervals, the blood allowed to clot at 37°C. for 1 hour and then centrifuged, and the serum was removed. The antibody titres of the sera were estimated by means of the hemolytic complement fixation test, using the method described by Fulton and Dumbell (1949) with drops of the fluids on perspex plates. The dilutions of the sera employed were 1/4, 1/8 and 1/16. The antigen for the test was prepared by extraction of the dried worms with saline, the lipoids having previously been removed by acetone and ether. The complement was obtained from guinea pigs. Five weeks after the first injection, little complement was fixed by any of the rabbits. At seven weeks, the two rabbits given 10

mgs. of antigen/kg. had high titres in a 1/4 dilution, and one of the rabbits given 20 mg./kg. had a similar titre. The rabbits given 1 mg./kg., and one of the rabbits given 20 mg./kg. fixed only small quantities of complement and their sera were not employed for further experimental work. The normal control rabbit serum fixed only insignificant amounts of complement. The sera were kept at a temperature of −2°C. until required, but were not stored for a long period.

Experiments were undertaken using these immune rabbit sera, *D. immitis* and *L. carinii* antigens, and the filarial infections which were available. It is regretted that homologous antigen and antiserum were not always available; *e.g.*, *D. immitis* was the only antigen available in quantity and *D. repens* the only parasite available in dogs. As reactions in filarial infections are of a group character (Rodhain and Dubois, 1931, 1932; Mohr and Lippelt, 1940), antigens as closely allied as the above would detect any antibodies present.

A. EXPERIMENTS *IN VITRO*

(1) *With infective larvae.* Infective larvae of *D. repens* and of the monkey filaria were obtained by allowing *Aedes aegypti* mosquitoes to feed on an infected animal anesthetized with sodium pentobarbital. When the larvae were mature, the mosquitoes were dissected in a drop of saline and the larvae were picked up with a fine capillary pipette and transferred to the serum to be tested for immune bodies, a drop or two of which had been placed on a sterile slide. The serum was then walled off with a ring of melted vaseline and a sterile cover glass was applied. Small quantities of penicillin and streptomycin were added to the preparations to inhibit growth of bacteria. The slides were kept at room temperature and examined at intervals.

Several series of experiments were performed using rabbit sera, and also dog and monkey sera prepared as described above with their respective infective larvae. No precipitates formed on the infective larvae in the immune sera. The larvae in these preparations survived for varying periods, but could live for approximately one week. The survival times of the larvae in immune and normal sera were not significantly different.

(2) *With adult worms.* The only adult filarial worms available were *L. carinii*. Males and females were incubated in Carrel flasks at 37°C. with immune and normal rabbit sera. They survived for 3–4 days just as well in serum from immune rabbits as in that from normal rabbits, and their motility was equally good in the two cases. No precipitates were observed round the worms. This result was confirmed by repeated experiments.

(3) *With microfilariae.* a). Microfilariae of *L. carinii* were incubated at 37°C. under sterile conditions with immune and normal rabbit sera in small glass flasks sealed with sealing wax, and they were examined at intervals with the inverted microscope according to the technique described by Hawking, Sewell and Thurston (1950). The survival times of the microfilariae in different experiments varied considerably; in some cases they lived for 7–8 days, while in others they died within 2–3 days. The nature of the serum, whether from nor-

mal or immunized rabbits, exerted no consistent influence however on the time of survival, nor were any other differences noted suggesting an immune reaction, *e.g.* agglutination of the microfilariae in the immune serum compared to the control. The microfilariae were taken out of the tubes after death and examined with a higher magnification than was possible with the inverted microscope. No differences were noted between those incubated with immune and those with normal sera.

b). Adhesin reactions. An attempt was made to demonstrate this phenomenon which was described by Pandit *et al.* (1929). (The serum to be tested for immune bodies is added to diluted citrated blood containing microfilariae; a positive test is shown by the white blood cells adhering to the microfilariae.) Sera from a dog whose peripheral blood had become free from microfilariae, from a dog whose peripheral blood contained microfilariae, from a normal dog and from immunized and normal rabbits, were tested against blood from a dog with a high microfilaria count. Only one or two leucocytes were seen adhering to microfilariae in one preparation and that had been made with the serum from the dog whose peripheral blood had once contained microfilariae, but the reaction was not sufficiently marked to be significant.

B. EXPERIMENTS *IN VIVO*

(1) *Passive immunity.* Two cotton rats infected with *L. carinii* were each given a total of 6 cc. of immune rabbit serum injected intraperitoneally in 9 and 10 daily doses respectively. Microfilaria counts were taken before and after the course of injections, and the rats were autopsied one week after the last injection. In both cases the microfilaria count decreased, but this could have been due to the variation found in the counts in *L. carinii* infections. In one rat which had a long standing infection, 55% of the adult females were dead, but in the second rat all the adults were alive at autopsy, so the result is not significant.

(2) *Active immunity.* a). Prophylactic value of *D. immitis* antigen. Ten normal cotton rats were given a course of injections of 20 mg./kg. of *D. immitis* antigen as described previously for the rabbits. Eight weeks after the first injection, sera (obtained by heart puncture of the rats) were subjected to the complement fixation test. Insignificant amounts of complement were fixed as compared to those fixed by the rabbits. The test was repeated three weeks later, and again only insignificant amounts of complement were fixed. The rats were then put into tanks along with normal untreated rats so as to expose them to infection by *L. carinii* as described by Hawking and Sewell (1948). After the incubation period, blood smears were taken from the immunized and control rats. Of the immunized rats, 7 out of 8 had microfilariae in their blood, and of the control rats, only 4 out of 10 had microfilariae, showing that the course of injections of antigen had had no prophylactic effect.

b). Therapeutic effect of *D. immitis* antigen. Three pairs of cotton rats infected with *L. carinii*, each pair having been infected at the same time and in the same tank, and all having microfilariae in their blood, were used for this

experiment. One rat of each pair was given a course of subcutaneous injections of *D. immitis* antigen (20 mg./kg.) every ten days for a total of nine injections. Weekly microfilaria counts were taken and the rats were autopsied one week after the last injection. The number of adults found was recorded and their lengths measured (Table 1). The worms of the immunized rats did not differ in state or length from those of the control rats. There was a great weekly variation in the microfilaria counts in both groups of rats but there was no general trend in either group.

TABLE 1

The effects of subcutaneous injections of D. immitis *antigen on* L. carinii *infections in cotton rats*

COTTON RAT	NUMBERS OF ADULT WORMS		AVERAGE LENGTHS OF ADULT WORMS (CM.)	
	Male	Female	Male	Female
2701 (Immunized)	21	18	3.3	8.2
2700 (Control)	3	2	3.4	6.9
2706 (Immunized)	27	21	2.6	9.4
2704 (Control)	17	17	2.7	7.8
2695 (Immunized)	61	41	2.1	7.5

TABLE 2

The size of wheals produced in dogs by intradermal injection of 0.05 cc. of D. immitis *antigen*

DOG NUMBER	SIZE OF WHEALS IN MILLIMETERS								
	10 minutes			20 minutes			30 minutes		
	D. immitis 1/2,000	*D. immitis* 1/8,000	Ringer*	*D. immitis* 1/2,000	*D. immitis* 1/8,000	Ringer*	*D. immitis* 1/2,000	*D. immitis* 1/8,1000	Ringer*
Infected									
3	10 x 15	12 x 13	7 x 8	19 x 19	15 x 16	7 x 7	20 x 20	17 x 18	6 x 8
10	14 x 16	12 x 15	7 x 8	18 x 18	17 x 17	7 x 9	19 x 20	18 x 18	8 x 8
11	10 x 12	10 x 12	9 x 11	13 x 13	13 x 13	10 x 10	13 x 13	13 x 15	10 x 10
Normal									
A	8 x 10	9 x 10	8 x 10	9 x 9	10 x 10	7 x 11	9 x 9	9 x 10	6 x 6
B	8 x 8	8 x 8	6 x 6	11 x 11	11 x 11	6 x 7	12 x 12	11 x 12	5 x 6
C	8 x 10	7 x 10	5 x 7	9 x 12	8 x 11	5 x 7	9 x 12	9 x 12	5 x 7

* All the wheals with Ringer were indistinct at 30 minutes.

Job No. 5317

C. SKIN TESTS

Skin tests were performed on the animals available, using *D. immitis* and *L. carinii* antigens. The dried worms were prepared as described above for the

immunization of rabbits. Ringer's solution was used as a control. Dogs, monkeys and rabbits were examined, but not cotton rats as their skin is not satisfactory for intradermal injection.

(1) *Dogs.* Three dogs (3, 10 and 11) infected with *D. repens* were examined. Dogs 10 and 11 had microfilariae in their blood; both were light infections and both had been infected on the same date. Dog 3 was an old infection and at the time of the skin tests microfilariae were no longer present in the blood. Three normal dogs, A, B, and C, were used as controls. The dogs were anesthetized with intravenous sodium pentobarbital, and the shaved skin of the abdomen was used for all tests. The dilutions of antigen used were 1/2,000 and 1/8,000 and 0.05 cc. of each was injected. The size of the wheal immediately after injection was measured and if it varied greatly from the average figure, the injection was repeated at a different site. Each set of injections was repeated more than once in each animal. Table 2 shows the measurements of the wheals produced in the dogs with *D. immitis* antigen. The wheals produced by *L. carinii* were of the same order. After 30 minutes the wheals gradually faded away. The wheals produced by Ringer's solution did not increase in size, but remained stationary at first and then gradually decreased. In all the dogs, infected and normal, the wheals produced by the antigen were well defined. Erythema is not recorded because of the difficulty of observing it on the dark skin of some of the dogs. In the three infected dogs, the wheals produced by the antigen were always larger than in any of the control animals; this was more marked with dogs 3 and 10, than with dog 11. As the smallest wheal in an infected animal (dog 11) approximated in size the largest wheal in a normal animal (dog B), evaluation of the test is difficult. With such a small number of animals it is not possible to deduce any limiting figures. No delayed reactions were observed.

(2) *Monkeys.* In monkeys with filariasis, it was not possible to produce well defined wheals. The wheals which were produced rapidly disappeared, and no immediate or delayed reactions were produced with the above dilutions of antigens, or with a 1/500 dilution.

(3) *Rabbits.* As with monkeys, it was not possible to produce well defined wheals and the injection of antigen intradermally into immunized rabbits produced no significant reaction compared to normal animals.

Immune sera from rabbits III, IV and VI were used in an endeavor to demonstrate a Prausnitz-Kustner reaction. One-tenth cc. of immune serum was injected intradermally into a normal rabbit and 30 minutes later 0.1 cc. of 1/500 *D. immitis* antigen was injected into the same site. The controls consisted of normal rabbit serum with antigen injected into the same site half an hour later, of each sera alone, and of the antigen alone. All these immune rabbit sera had been shown to have a high titre of complement fixing antibodies. Serum from rabbit III + antigen produced no reaction; serum from rabbit IV + antigen produced a wheal with pseudopodia of 3 x 4.5 cms. at 5½ hours which had disappeared at 23 hours, while the only control substance producing a reaction was the immune serum alone which produced a wheal 2 x 2 cms. Serum from rabbit VI + antigen produced a wheal measuring 3.5 x 2 cms. at 5 hours which disappeared at 22 hours, while that produced by the normal

serum and antigen measured 2.5 x 1.5 cms. These reactions are not very striking, but they provide a slight indication of the presence of antibodies in the sera of rabbits IV and VI.

HISTAMINE REACTIONS IN DOGS, MONKEYS AND RABBITS

In view of the failure to demonstrate immediate skin reactions in monkeys and rabbits, their reactions to intradermal injection of histamine were investigated. Five-hundredths cc. of histamine acid phosphate in dilution of 1/1,000,000, 1/100,000, 1/10,000 and 1/1,000 were each injected intradermally into a dog, a monkey, and a rabbit. In the dog, all dilutions produced wheals which rapidly increased in size and which were similar to those produced by the injection of antigen. In the monkey and rabbit the wheals produced by the injection of the fluid gradually faded. The immediate response to antigen injected intradermally into an immune animal is believed to be due to the liberation of histamine. The results described above are in accordance with this belief, since responses were obtained to antigen in dogs, which react to histamine with wheal formation, but not in monkeys and rabbits, which do not react to histamine.

DISCUSSION

It is almost certain that filarial infections produce some kind of immune response in the host, since

1. Practically all kinds of infections produce immune responses.
2. In populations exposed to heavy filarial infections, the infection rate does not always increase *pari passu* with age, although the highest rates are often found in the older age groups.
3. There is much evidence that filarial lymphangitis and Calabar swellings are antigen-antibody reactions.

However, the demonstration of specific immune reactions in the laboratory has proved difficult. The possible ways for demonstrating such reactions in the host—parasite relationship may be summarized as follows (after Culbertson, 1941):

I. *With the host.*
   a). Exposure of the host to reinfection and demonstration of its ability to resist infection.
   b). Injection of antigen intradermally, and demonstration of immune reactions in the form of wheals, edema, and erythema.

II. *With serum from the host.*
   a). Passive transfer of immunity by the injection of the host's serum into a nonimmune animal.
   b). Tests to detect any of the following immune responses: agglutinins, precipitins, complement fixing antibodies, adhesins.

III. *With the Parasite.* Demonstration in the immune host of
   a). A smaller number or smaller size of parasites developing.
   b). The failure of maturation of parasites.
   c). A briefer persistence of the parasites.

Analyzed according to the above summary, the facts about immune responses to filarial infections, as shown by the above experiments and from the literature, may be summarized as follows:

I. *Reactions in the host.* In the above experiments, cotton rats "immunized" by injections of *D. immitis* antigen developed a higher infection rate when exposed to *L. carinii* than the control rats. In unpublished experiments in the National Institute for Medical Research, Miss W. A. F. Webber found that cotton rats, infected with *L. carinii* and then treated with cyanine and Hetrazan to kill the adult worms and microfilariae, became as heavily infected on re-exposure as the normal cotton rats.

The skin test as a diagnostic aid in filariasis has been the subject of numerous papers since it was described by Taliaferro and Hoffman (1930) and Fairley (1931) using *D. immitis* antigen. The literature has been reviewed by Saunders *et al.* (1946). The significant percentages of false positives and false negatives render the specificity of the skin test doubtful and Lampe (1950) after a careful investigation with *D. immitis* antigen concluded that the test "cannot serve to arrive at a diagnosis of clinical wuchereriasis". In the present experiments, 3 dogs infected with *D. repens* gave mild positive skin reactions; infected monkeys gave no reaction; and hyperimmunized rabbits gave no direct reaction and only a mild Prausnitz-Kustner reaction.

If *Aedes aegypti* with infective larvae of *D. repens* are allowed to feed on five day old mice, infective larvae can persist at the site of inoculation for at least 3 days without provoking local signs of reaction. This would tend to support the suggestion of Augustine and L'herisson (1946) that many of the false positives in human skin tests may be due to sensitization to nonhuman filarial worms which fail to establish themselves.

II. *Reactions with serum.* No evidence could be obtained of any passive transfer of immunity by injection of serum from an immunized animal. Experiments with microfilariae and infective larvae in immune sera *in vitro* gave no indication of the presence of agglutinins, precipitins or adhesins, such as have been demonstrated with certain nematode larvae, and no differences were noted in the survival times of the larvae.

The complement fixation test described by Fairley (1931) has been used in laboratories as an aid to the diagnosis of filarial infections. In the present experiments, repeated injection of *D. immitis* antigen produced high titres of complement fixing antibodies in rabbits but not in cotton rats. Tests employed for some time in this laboratory have been successful in demonstrating complement fixing antibodies in cotton rats infected with *L. carinii* and in a number of human cases, but difficulties are encountered with filarial infections in monkeys and dogs. Since it was learned that Dr. W. Minning of the Tropeninstitut, Hamburg, is conducting an investigation of this subject, my own experiments were discontinued and a collaboration was arranged with his laboratory, the results of which will be published by Dr. Minning in due course. Complement fixation tests in monkeys and dogs often produce incongruous results, with some negative reactions in long-standing, heavy infections, and occasional

positives in uninfected animals. The explanation of these anomalies is not yet known. In general, other investigators have usually succeeded as I have, in producing complement fixing antibodies in rabbits with a variety of filarial antigens, but results in other animals have been negative or undependable.

As regards clinical results, Fairley (1931, 1932) reported a high percentage of positive reactions in cases of *W. bancrofti* and *Loa loa* infections, but others have had varying success with antigens prepared from *D. immitis*, *L. carinii* and *Onchocerca volvulus*. Thus, while complement fixing antibodies can be demonstrated in a number of cases of filariasis, there is the same overlapping of positive and negative results as in the skin test.

In the present experiments the survival time of adult filarial worms, microfilariae and infective larvae in immune serum, regardless of the presence of any of the above reactions, was not significantly different from that in normal serum. These observations differ from those of Arnold and Duggan (1937) who found that sera of rabbits immunized with *D. immitis* antigen had a markedly lethal effect on microfilariae of that species; and of Murata (1939) who reported that the survival time of microfilariae transfused into immunized rabbits was somewhat shorter than in the controls. This is difficult to assess as 90 per cent of microfilariae transfused into an animal quickly disappear (Hawking 1940).

III. No experiments on the development of filarial worms in host animals were attempted in view of the lack of knowledge about important stages of the intrinsic cycle, and the difficulty in many species of locating adult worms at autopsy.

GENERAL CONCLUSION

The results described in this paper and those reviewed above can now be considered in their general bearing on the host-parasite relationship. The ideal of the parasite is to live and let live and the more closely this state of mutual toleration is reached, the more successful is the parasite. In the same way, the more perfect the adaptation of the parasite, the less is the immune response which it provokes. Other helminths, *e.g. Nippostrongylus*, *Ancylostoma* or *Schistosoma*, cause appreciable lesions and the immunological reactions which they provoke are readily manifested. In the case of filarial infections (as is shown by the above work) immune responses are difficult to demonstrate and are slight in extent. On the other hand, the filarial worms are known to be highly successful in their adaptation to their hosts, since most species of these helminths produce only mild and infrequent pathological lesions. The particular filariae studied in the present work are especially well adapted as is shown by the absence of harmful effects. Thus *D. repens* in the dog and the new dirofilarial worm in the monkey cause no appreciable reaction either to the adult worms or to the microfilariae, and *L. carinii* lives in the pleural cavities of the cotton rat without producing more than a mild cellular exudate. Accordingly the failure to demonstrate well-marked immunologic reactions to filarial infections, although at first sight disappointing, is really in accordance with the general

principle described above, that the more perfect the adaptation of the parasite, the less is the immune response of the host.

SUMMARY

An investigation was made of the immune responses produced in three laboratory filarial infections: *Dirofilaria repens* in dogs, a species of filarial infection in monkeys and *Litomosoides carinii* in cotton rats. Immune sera with a high titre of complement fixing antibodies were obtained by immunizing rabbits with *Dirofilaria immitis* antigen.

These immune sera and sera from infected animals showed only a slight or no immune reactions *in vitro* against infective filarial larvae, against adults of *L. carinii* and against microfilariae. Immune serum had no therapeutic action upon infections by *L. carinii*. Agglutinins, precipitins and adhesins could not be demonstrated. *D. immitis* antigen had no prophylactic or therapeutic action upon infections by *L. carinii*. Weak immediate skin reactions were obtained in dogs infected with *D. repens*, but not in any of the other animals. A doubtful Prausnitz-Kustner reaction was observed with immune rabbit sera.

It is concluded that filarial infections do produce a certain immune response but this is small in extent and difficult to demonstrate. The smallness of the immune response may be correlated with the great success of filarial parasites in adapting themselves to their hosts, and it is a good example of the general principle that the more perfect the adaptation of the parasite, the less is the immune response of the host.

ACKNOWLEDGMENTS

This work was carried out while holding a Colonial Research Studentship. Grateful acknowledgments are due to Sir Charles Harington, F.R.S., for providing facilities at the National Institute for Medical Research, to Dr. F. Hawking for guidance and advice, to Miss W. A. F. Webber for the supply of cotton rats infected with *L. carinii*, and to Sir Neil Hamilton Fairley, F.R.S., for a supply of *D. immitis* antigen.

REFERENCES

ARNOLD, J. G., JR., AND DUGGAN, T. L. 1937, Immunological studies with *Dirofilaria immitis*, *J. Parasit.* **23:** 561.

AUGUSTINE, D. L., AND L'HERISSON, C. 1946, Studies on the specificity of intradermal tests in the diagnosis of filariasis, *Amer. J. Hyg.* **43:** 38–40.

CULBERTSON, J. T. 1941, Immunity against animal parasites, New York, Columbia University Press.

FAIRLEY, N. H. 1931, Serological and intradermal tests in filariasis. A preliminary report, *Trans. R. Soc. Trop. Med. Hyg.* **24:** 635–648.

FAIRLEY, N. H. 1932, The skin test and complement fixation reactions in filariasis, *Trans. R. Soc. Trop. Med. Hyg.* **25:** 220–221.

FULTON, F., AND DUMBELL, K. R. 1949, The serological comparison of strains of influenza virus, *J. Gen. Microbiol.* **3:** 97–111.

HAWKING, F. 1940, The transference of microfilaria bancrofti into natural and unnatural hosts, *Ann. Trop. Med. Parasit.* **34:** 121–129.

HAWKING, F., AND SEWELL, P. 1948, The maintenance of a filarial infection (*Litomosoides carinii*) for chemotherapeutic investigations, *Brit. J. Pharmacol.* **3:** 285–296.

HAWKING, F., SEWELL, P., AND THURSTON, J. P. 1950, The mode of action of hetrazan on filarial worms, *Brit. J. Pharmacol.* **5:** 217–238.

LAMPE, P. H. J. 1950, Study on filariasis in Surinam, *Docum. Neerl. et Indones. Morbis. Trop.* **2:** 193–208.

MOHR, W. & LIPPELT, H. 1940, Bericht über weitere ergebnisse mit der filarienkomplementbindungs-reaktion, *Klin. Wschr.* **19:** 157–159.

MURATA, H. 1939, Immunologische studien über *Dirofilaria immitis*, *Fukuoka Acta Med.* **32:** 54.

PANDIT, C. G., PANDIT, S. R., AND IYER, P. V. S. 1929, The adhesion phenomenon in filariasis: a preliminary note, *Ind. J. Med. Res.* **16:** 946–953.

RODHAIN, J., AND DUBOIS, A. 1931, Cases of parasitism by *nochocerca volvulus* in Europeans. Skin reactions—allergy, *Rev. Belge Sci. Med.* **3:** 613–623.

RODHAIN, J., AND DUBOIS, A. 1932, A contribution to the study of intradermal reactions in human filariasis, *Trans. R. Soc. Trop. Med. Hyg.* **25:** 377–382.

SAUNDERS, G. M., BIANCO, A. A., AND JORDAN, W. S. 1946, Intradermal tests with "*Dirofilaria immitis*" antigen as a diagnostic aid in human filariasis, *Nav. Med. Bull.*, Wash. **46:** 1242–1253.

TALIAFERRO, W. H., AND HOFFMAN, W. A. 1930, Skin reactions to *Dirofilaria immitis* in persons infected with *Wuchereria bancrofti*, *J. Prev. Med.* **4:** 261–280.

TERRAMYCIN IN SCHISTOSOMIASIS: A TRIAL IN SIX CASES

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In view of certain unconfirmed and conflicting reports on the successful use of terramycin in schistosomiasis in the Union of South Africa, and of claims advanced elsewhere as to its value in treating the disease, we have tried to determine its effectiveness in a carefully conducted trial.

TABLE 1

Results of treating 6 cases of schistosomiasis with terramycin

DATE	AGE	SEX	DOSE	INFECTION	FOLLOW-UP
15.1.52	Adult	F	30 gms.	*S. mansoni*	6.2.52 +
					13.2.52 ±
					15.2.52 +
					21.2.52 +
18.1.52	10 yrs.	M	24 gms.	*S. haematobium*	23.1.52 +
					31.1.52 +
					7.2.52 +
					13.2.52 +
					15.2.52 +
19.1.52	Adult	F	25 gms.	*S. haematobium*	23.1.52 −
					31.1.52 +
					7.2.52 +
					14.2.52 +
11.12.51	9 yrs.	M	22.5 gms.	*S. mansoni*	4.1.52 +
					17.1.52 +
					23.1.52 +
					31.1.52 +
					7.2.52 +
					14.2.52 +
22.12.51	5 yrs.	F	12.5 gms.	*S. mansoni*	27.12.51 +
					4.1.52 −
					18.1.52 +
					23.1.52 +
17.12.51	Adult	M	25 gms.	*S. mansoni*	27.12.51 +
					4.1.52 −
					17.1.52 +
					23.1.52 +
					31.1.52 +

The cases (6 in all) were infected with either the urinary (*Schistosoma haematobium*) or intestinal (*S. mansoni*) parasite. Care was taken to ensure that each patient had an obvious infection with viable ova in the excreta before any treatment was commenced. The cases could be labeled from the laboratory aspect as moderate or moderately severe, as none passing only a few or scanty ova was

selected. Since terramycin can be taken at all ages without serious constitutional disturbance, we included children as well as adults. All the cases were confined to hospital, none being permitted to leave at any time. There was therefore, no possibility of reinfection by exposure.

When treatment was begun, the patients did not receive any other drug, nor was the treatment preceded by a drug which might have had a lethal effect on the helminth. The cases were kept under observation and weekly specimens studied. Each patient received 2 capsules each (250 mg.) three times a day—at 10 a.m., 2 p.m. and 6 p.m.—for about 16 days, except the female child aged 5 years who had a total of 50 capsules over 8 days. If after 4 weeks the patient still continued to pass viable ova, he was considered as not being cured.

Urine to be examined for ova was allowed to stand in a conical flask for half an hour, and a large part of the supernatant fluid was then discarded. The remainder was centrifuged at 1000 r.p.m. in two tubes, and the supernatant was again discarded. To the deposit in one tube, warm (30°C.) distilled water was added and the contents were examined after 30 minutes for miracidia. The deposit from the other tube was examined microscopically for ova.

For stool examination, scrapings were made from the surface of the formed stool, any mucus or blood present being expressly included. These were emulsified in warm water and allowed to stand in a conical flask for 15 minutes, when the supernatant fluid was discarded and the sediment resuspended in fresh water. After a third washing similar to this, aliquot portions were centrifuged and one was examined for miracidia with a hand lens, while the other was examined microscopically for ova. The results are presented in tabular form.

It will be seen from these results that terramycin did not cure any of the six cases in which treatment and follow-ups were completed.

SUMMARY

Five cases of schistosomiasis were unsuccessfully treated with terramycin in doses ranging from 1½ gm. daily for a period of approximately 16 days. The sixth case, a child aged 5 years who received 1½ gm. daily for 8 days, was also uncured.

ACKNOWLEDGMENTS

We would like to record our thanks to Messrs. Petersen Ltd. for the supply of terramycin and to Dr. R M. Morris O.B.E., M.D., Secretary for Health, Southern Rhodesia, for his kind permission to publish this paper.

XENODIAGNOSTIC TECHNIC AS AN AID IN DIAGNOSIS OF TRICHINOSIS[1]

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-Hall and Collins (1937), Nolan and Bozicevich (1938) and Wright *et al.* (1943) have shown that the digestion-Baermann method and the technic of microscopic examination of 1 gm. of compressed tissue for the detection of *Trichinella spiralis* larvae in muscle tissue have definite limitations and supplement each other. The first authors have stated that microscopic examination alone will probably not detect cases of light infection involving either live or dead trichinae while the digestion method will detect the presence of even small numbers of live trichinae rather dependably; but, since the efficiency of the Baermann apparatus depends for its effect on the movement of live worms and the effect of gravity in bringing down these moving worms, the digestion method is of little value for the detection of dead trichinae.

Kerr *et al.* (1941) reported that compression alone was effective in detecting 90 per cent of infections with dead larvae. Wright *et al.* (1943) stated that, of 855 positive cases found among 5313 human diaphragms, the direct microscopic examination of 1 gm. of muscle detected 70.5 per cent, while the digestion-Baermann technic for the examination of the remainder of the diaphragm detected only 62.6 per cent, a difference due probably to a predominance of cases with only dead larvae.

Jacobs (1938) also pointed out that the two technics supplement each other, the digestion method detecting live larvae in almost any number, the compression method mainly dead larvae down to about 1 larva per gm. of tissue. Examination of 10 gm. samples by the compression method by Jacobs revealed 6 per cent additional positives among 100 diaphragms negative by the routine methods.

Gould (1945) reported an incidence of 29.3 per cent in 317 cases examined by compression of 10 gm. samples as against an incidence of 19.7 per cent in 1031 cases by compression of 1 gm. samples.

Mazzotti and Pastrana (1943), in a study of trichinosis in Mexico, reported that calcified cysts with dead larvae are frequently destroyed by the digestion-Baermann method, resulting in a reduction in the number of cysts recovered. They noted that results frequently are negative by this method while a smaller sample of muscle under compression may show cysts. They concluded that direct examination of muscle tissue should be made on larger than 1 gm. samples.

Mazzotti (1944) reported 12 per cent of 473 diaphragms examined by compres-

[1] The author wishes to express his appreciation to Dr. Luis Mazzotti, Chief of the Dept. of Helminthology, for his helpful suggestions and criticisms.

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sion of 10 gm. samples to be positive; the digestion of the remaining negatives revealed no more positives. He concluded that the compression method using 10 gm. of diaphragm appeared to be more effective than the commonly used combination of examining 1 gm. of muscle by compression with digestion of the remainder, as previously described.

MATERIALS AND METHODS

In view of the observations of Mazzotti (1944), it was decided in this investigation to examine human diaphragms by compression only, using larger than

TABLE 1

Nature of positive cases among 100 human diaphragms examined for Trichinella spiralis *by the compression method*

WT. OF DIAPHRAGM IN GMS.	RESULTS	XENODIAGNOSIS
104	1 cyst—heavily calcified	—
100	167 cysts* (1⅔ cysts per gm. of tissue) various stages of calcification	Pos.
145	5 cysts—all heavily calcified	Neg.
195	1 cyst—heavily calcified	Neg.
50	20 cysts—various stages of calcification	Pos.
65	1 cyst—heavily calcified	Neg.
60	18 cysts—heavily calcified	Neg.
95	5 cysts—moderately calcified	Neg.
75	4 cysts—moderately calcified	Neg.
56	2 cysts—heavily calcified	Neg.
60	120 cysts* (2 cysts per gm. of tissue) various stages of calcification many heavily calcified	Pos.
51	4 cysts—moderately calcified	Neg.
50	1 cyst—slightly calcified	Neg.
75	10 cysts—various stages of calcification	Pos.
58	87 cysts* (1½ cysts per gm. of tissue) various stages of calcification	Pos.

* Estimated total count based on the determination of number of larvae found in a random 10 gm. sample.

10 gm. samples and, when possible, to use the entire diaphragm. A study was made of 100 diaphragms taken from autopsies in two hospitals in Mexico City with the purpose of determining the nature and degree of infection of the positive cases. Xenodiagnosis, using albino rats, was employed as a method of determining the viability of the larvae in the positive cases.

Attempts were made originally to secure entire diaphragms for microscopic study. Due to the medico-legal aspects of most of the cases only smaller portions were obtainable, but all samples studied weighed 50 gm. or more.

Prior to examination, the fascia was carefully dissected from both sides of the diaphragm, thus facilitating the spreading of the muscle fibers during compression. Portions of muscle up to 5 gm. were rendered compressible and suitable

for study in one examination. All examinations were made under the low power of the dissecting microscope, with higher magnifications used when needed. Questionable cysts were teased from the muscle and studied separately.

Compression of the muscle was obtained by placing samples between two pieces of plate glass (20 cm. × 8 cm.) fitted to a wooden frame and held secure by bolts on either side, similar to trichinoscopes depicted by Gould (1945).

All but one of the positive diaphragms (one in which only a single heavily calcified cyst was found) were fed to rats free of *T. spiralis* infection. These rats were sacrificed after a period of one month or more. Those animals showing no larvae in their diaphragms by the compression technic were digested in their entirety by the digestion-Baermann method and an examination for larvae made.

RESULTS

From a study of Table 1 it can be seen that there is a very high incidence among positives (80 per cent) of low grade infections of the order of less than 1 cyst per gm. of tissue. Only 2 of these cases contained viable cysts. Sixty per cent of the positives were of the order of less than 1 cyst per 10 gm. of tissue, with none of these cases showing viable larvae.

DISCUSSION

From the results above it can be seen that if the older routine method of examining 1 gm. of tissue microscopically with digestion of the remainder had been employed there is no doubt that a majority of the cases would have been missed for this technic misses dead larvae of the order of less than one per gm. (Wright *et al.*, 1943)

Hall and Collins (1937) noted, in their study of those cases showing live trichinae, that light infections predominated. They expressed the possibility that trichinae in heavy infections may calcify and die much more rapidly than trichinae in light infections, as evidenced by the fact that dead trichinae predominated in heavy infections found. This view is not supported by the present evidence since the heaviest infections found contained viable larvae and the lighter ones only dead larvae. However, in this study, infections were generally lighter than those reported by Hall and Collins. A possible explanation for these lighter infections may lie in the difference in economic status of the two countries in question. While many hogs in the United States are garbage fed, and the higher level of living results in more pork scraps being present in such garbage, in Mexico little if any garbage is fed to hogs. Such garbage as might find its way to these animals is in general very low in meat scraps. Hogs frequently depend upon scavenging on human feces for sustenance.

Previous workers have repeatedly differentiated living from dead infections on the basis of the microscopic appearance of the larvae, together with the information obtained from the digestion technic. With such information it has been thought possible to differentiate viable infections of recent origin from dead infections of longer duration. Under such circumstances the digestion-Baermann

method might be comparable to any other helminthic survey technic in which only viable infections are detected. It has been the experience in this investigation however that, microscopically, live larvae could not be detected with any degree of certainty in those cases which by xenodiagnosis were shown to be viable. Thus the detection of viability must rest upon some other method than microscopy.

Jacobs (1938) stated that the digestion-Baermann method will detect living larvae in almost any number, but it has been the experience in this laboratory that such is not the case. Through the use of xenodiagnosis it is believed a greater degree of accuracy in determining viability can be obtained. Theoretically only one viable male and one viable female worm would need to be ingested by a susceptible host to establish an infection. Doerr and Menzi (1933) state that they have established such infections in rats. Roth (1938) demonstrated by direct counting that a female trichina produces about 1630 encysted larvae in muscle, thus indicating the ease and certainty with which an infection, once established, might be detected by xenodiagnosis.

In order more accurately to evaluate the data obtained in future survey work it is proposed that subsequent to microscopic examination of the entire diaphragm, the tissue be fed to a noninfected susceptible host. From the data obtained from both microscopic examination and xenodiagnosis an index of the incidence of infection more accurate than any obtained to date can be obtained. Xenodiagnosis of all samples will serve as a check for any light living infections possibly overlooked in the microscopic examination. From the study of the data obtained from microscopic examination alone the nature of the infections may be determined, i.e. whether heavy or light, degree of calcification and estimation of approximate duration of infection. From a study of the data obtained from xenodiagnosis the incidence of current viable infections may be plotted and compared with other viable helminth infections. Repeated surveys of this nature over a period of years would give an index of the rise or fall of active and passive infections.

SUMMARY

A study was made of 100 human diaphragms in Mexico City to determine the incidence and degree of infection with *T. spiralis*.

Direct microscopic examination of samples weighing 50 gm. or more revealed a very high incidence (15 per cent) of low grade infections. Twelve of the 15 positive cases showed less than 1 cyst per gm. of tissue, while 9 showed less than 1 cyst per 10 gm. of tissue.

Xenodiagnosis, using albino rats, was used to detect viability in the positive cases. This method is proposed as a means of detecting all cases having viable larvae in future survey work.

REFERENCES

1. Doerr, R., and Menzi, E. 1933, Vergleichende Untersuchungen über die Empfänglichkeit der Ratte und des Meerschweinchens für die Infektion *per os*, *Zentralbl. f. Bakt., I.*, Orig. **128:** 177–188.

2. Gould, S. E. 1945, Trichinosis. Charles C Thomas Publ. Springfield, Illinois.
3. Hall, Maurice C., and Collins, Benjamin J. 1937, Studies on trichinosis. I. The incidence of trichinosis as indicated by post-mortem examination of 300 diaphragms, *Pub. Health Rep.* **52:** 468–490.
4. Jacobs, L. 1938, Studies on trichinosis. X. The incidence of light infestations of dead trichinae in man, *J. Wash. Acad. Sci.* **28:** 452–455.
5. Kerr, K. B., Jacobs, L., and Cuvillier, E. 1941, Studies on trichinosis. XIII. The incidence of human infection with trichinae as indicated by post-mortem examinations of 3,000 diaphragms from Washington, D. C., and 5 eastern seaboard cities, *Pub. Health Rep.* **56:** 836–855.
6. Mazzotti, L. 1944, Examen de 400 diafragmas humanos en la ciudad de México para investigar triquinosis, *Rev. Inst. Salub. y Enf. Trop.* **5:** 157–161.
7. Mazzotti, L., y Pastrana, A. 1943, La investigación de triquinosis en tejidos musculares por el método de digestión, *Rev. Inst. Salub. y Enf. Trop.* **4:** 337–342.
8. Nolan, M. O., and Bozicevich, John 1938, Studies on trichinosis. V. The incidence of trichinosis as indicated by post-mortem examinations of 1,000 diaphragms, *Pub. Health Rep.* **53:** 652–673.
9. Roth, H. 1938, Experimental studies on the course of trichina infection in guinea pigs. I. The minimum dose of Trichina larvae required to produce infection of the muscles; with an account of the potential productiveness of the female Trichina, *Am. J. Hyg.* **28:** 85–103.
10. Wright, W. H., Kerr, K. B., and Jacobs, L. 1943, Studies on trichinosis. XV. Summary of the findings of *Trichinella spiralis* in a random sampling and other samplings of the population of the United States, *Pub. Health Rep.* **58:** 1293–1313.

PERSISTENCE OF HOOKWORM LARVAE IN SOIL

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Infective hookworm larvae normally migrate vertically as high as moisture on uninterrupted surfaces permits and therefore during and immediately following rains they tend to accumulate at or above the surface of the soil (Augustine, 1922, 1923b). Under natural conditions they have been found to persist in soil and in laboratory cultures for various periods up to several months. However, the majority of the larvae usually die within a short period after the infective stage is reached. Exhaustion of stored food granules and desiccation have been regarded as the chief lethal factors (Cort, 1925a). The rapid early reduction of larvae in cultures was studied by Augustine (1923c) who observed that on drying soils some of the larvae escape desiccation by retreating ahead of the receding zone of free moisture but that a significant proportion of them remain at the surface and become victims of desiccation. This observation suggested that alternate wetting and drying would hasten the death of larvae and thus shorten the period of soil infectivity. However, the results of experiments which have most directly tested this hypothesis, while they support it, have not been sufficiently conclusive and quantitative to serve as a basis for investigations of related problems (Augustine 1923a, c; Cort 1925b). A major difficulty encountered in the earlier studies was the problem of distinguishing between the mortality of larvae and the degree of inefficiency of the technic (Baermann) used for isolating larvae from soil. The percentage of larvae that can be recovered with the Baermann apparatus is somewhat unpredictable and from some types of soil the yields are always low (Cort *et al.*, 1922; Cort, 1925a, b). It was of interest, therefore, to study the persistence of infective-stage hookworm larvae in relation to moisture fluctuation, using an improved technic of isolating the larvae from soil.

MATERIALS AND METHODS

The technic employed for the isolation of larvae from soil has been in use with various modifications since 1943. Basically, it involves an absorbent cotton pad which, when dampened and placed in close contact with moist soil, collects the larvae as they migrate upwards. The pad is then suspended in water in a conical flask or funnel and the larvae are collected from the sediment in much the same manner as with the Baermann apparatus. For the present study, circular pads 16.5 cm. in diameter were prepared from 16 layers of grade 50 absorbent cheesecloth, sewed loosely around the margin and at the center. Flexible, translucent plastic disks, 17 cm. in diameter, were used as covers for the pads. Larvae-laden pads were transported in metal mailing tubes. Conical 280 cc. beer glasses (Pilsener), calibrated at 50, 100 and 200 cc. levels, served as sedimentation flasks. Other materials employed were forceps for handling the pads, wide-bore 1 cc.

pipettes for taking samples from the flasks, shallow depression slides for microscopic study of the samples, and 1 per cent potassium iodide saturated with iodine for killing and staining the larvae.

The procedures were as follows: (1) Water was added to the soil until it was well moistened. (2) A pad was dampened, but not saturated, with water, pressed firmly over the soil and covered. (3) After 30 minutes, the pad and cover together were gripped at the margin with forceps, twisted into a tight roll with the cover on the outside, placed in a mailing tube, and enough water was added to keep the pad moist en route to the laboratory. (4) The pad was gripped with forceps at its center and withdrawn, leaving the cover in the tube. The pad was then forced into a sedimentation flask which had been filled with tepid water to

FIG. 1. Units of the isolating apparatus and the containers used for transport of the larvae-laden pads.

near the 200 cc. mark, placing the surface that had been in contact with soil uppermost so that, when in place, the pad assumed the shape of a fluted cone enclosing the contaminated surface (Fig. 1). The tube and cover were then rinsed several times with 10 to 15 cc. of water and after adding the rinse to the flask the pad was forced down to just below the water level and allowed to stand one hour or longer. (5) The yield could be judged roughly by inspection of the sediment. If the quantity was small, all of it was transferred to a depression slide. If the sediment contained a large mass of organisms, the pad was removed. Then, depending upon the anticipated yield of larvae (quantity of sediment), water was withdrawn to an appropriate level, air was bubbled into the flask to give an even suspension of the larvae and one or more 0.5 cc. samples were transferred to depression slides for microscopic study. (6) After adding just enough iodine to kill and lightly stain the organisms, the hookworm larvae were identified and counted.

Cultures were set up in a shaded area where the soil was typical of the coastal

plain of Georgia, being about 90 per cent fine sand with variable amounts of silt and organic elements but without appreciable clay. Bottoms were removed from metal buckets of the same diameter as the sampling pads (16.5 cm.), and 19 cm. high. With as little disturbance as possible to the soil, they were pressed into the ground to a depth of about 15 cm., placed side by side in two rows of 10 each. For protection against rain and other disturbances, an inverted-trough roof was provided which covered all of the buckets and rested on their rims. After thoroughly wetting the soil enclosed by 18 of the buckets on two successive days, a watery fecal suspension containing approximately 50,000 hookworm eggs was poured on the surface of each and covered with soil to a depth of 0.5 cm. The other 2 units were used later for observations on larvae from laboratory cultures. For five days, while larvae were developing to the infective stage, all cultures were thoroughly dampened daily, water being added in small amounts until it stood at the surface for one or two minutes. Daily sampling of one culture was started on day 4 but larvae were first recovered from it on day 5. Thereafter, only 9 of the cultures (to be referred to as the wet series) were dampened daily, while the other 9 (dry series) were dampened only when sampled. In the dry series, a culture was first sampled on day 9. In both series, an additional culture was sampled daily until all had been included. After the sampling was started on each culture it was continued daily until negative results were obtained on two successive days.

RESULTS

The initial sampling recovered 64 to 96 per cent (average 82%) of the total yields from individual cultures, except in the first instance (day 5) when many larvae were lost as a result of partial desiccation of the pad en route to the laboratory. After the cultures had been sampled three times, almost all of the larvae (average 99%) had been recovered. However, larvae were found in one culture for eight days. The average was 4.4 days.

In the wet series of cultures the yields diminished rapidly after day 8, the last culture (day 13) yielding only 2,900 as compared with a maximum of 46,200 and an average of 31,000 for the first 4 samplings. In the dry series, there was also progressive reduction in yields. However, the yields were greater from the dry cultures which were initially sampled on days 16 and 17 than from those of the wet series which were initially sampled on days 9 and 10. Of particular interest are the five paired cultures of the two series which were initially sampled simultaneously. Yields were respectively 2.9, 3.0, 5.7, 6.0 and 7.3 times greater from the dry series than from the corresponding cultures of the wet series (days 9–13, Fig. 2).

Second samplings in the wet series yielded an average of only one-third as many larvae as first samplings taken from other cultures on the same days. For that reason, it is clear that the pads removed many more larvae than were lost from the cultures in the same period as a result of other factors. It is to be noted, however, that the pads were protected from drying and they, in turn, protected the soil surface for one-half hour, whereas the unsampled cultures at the same time were afforded no protection against evaporation.

The cultures of the dry series had lost all evident moisture from the surface before the first one was sampled and the soil became dry and loose to a depth of about 0.5 cm. toward the end of the experiment. Presumably desiccation of larvae within the soil was chiefly responsible for the gradual reduction in the yields. Although the cultures of the wet series were dampened only once daily and the water soaked into the soil promptly, in all of them the surface retained a moist appearance between wettings throughout the period of the experiment. The results show, however, that the soil was dry enough to kill any larvae that remained at the surface and that, although the surface appeared to be damp, there probably was not enough moisture to permit migration to the surface for more than a few minutes after each wetting.

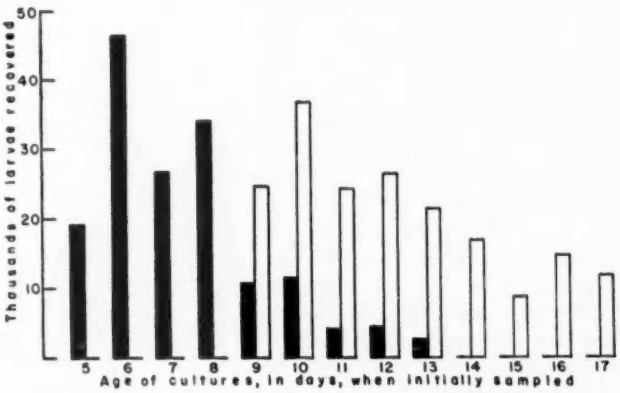

FIG. 2. Total yields of infective hookworm larvae from soil cultures that were inoculated with 50,000 eggs each. The nine cultures illustrated by solid bars were dampened daily; after day 5, those of the dry series (open bars) were dampened only when sampled. Sampling of the first culture of the wet series was started on day 5 and continued at daily intervals until negative. On successive days and in like manner sampling of an additional culture of the wet series was started. Sampling of the dry series was started on day 9 and carried out similarly.

On two thoroughly dampened units, like those used for the above cultures, suspensions of equal but undetermined numbers of larvae were poured onto the soil surface. One was sampled as soon as all water from the suspension had soaked into the soil and 24,800 larvae were recovered from it. The other was initially sampled 24 hours later and only 9,000 larvae were recovered, a reduction of approximately 60 per cent. Sampling was continued on both cultures daily until negative on two successive days. The respective total yields were 25,000 and 11,500. It is evident that when placed on the surface, less than half of the larvae migrated, percolated, or by both means got into the soil where they were protected against desiccation.

DISCUSSION

Previous observations on hookworm larvae in alternating moist and drying soils have been reported by Augustine (1923a, b). In his experiments on the varia-

tion in survival of larvae cultured from stools of twenty-two different individuals, infective larvae were placed in small (70–75 gm.) units of sandy loam, stored in moderate shade and dampened every 2 to 3 days. At weekly intervals the larvae from one unit of each series were isolated by means of the Baerman apparatus. In another series of experiments on various factors influencing the survival of larvae, similar units were used but the soil was kept constantly moist. In the dry-wet series the average reduction in larvae during the first week was 86 per cent (17–99%), the second week 93 per cent (67–99.5%) and within 7 weeks all larvae had died in 16 of the 22 series of units. The longest survival was between 7 and 8 weeks. In the second study there was one series of 10 constantly moist units which were stored in moderate shade as in the above experiment. Results were not markedly different in the two studies. There was 67 per cent reduction in 5 days, 89 per cent in 10 days and 92 per cent in 15 days. Only 1 surviving larva was found at 44 days and none at 7 weeks.

In experiments on the effect of burial, Cort (1925b) compared the yields of larvae from shaded and unshaded clay-loam units that were dampened daily, with similar units that were not dampened at all. Because the surface of the shaded-dampened units appeared to be constantly moist it was regarded as unlikely that desiccation was responsible for the total yield being only about half that from the corresponding undampened units. It now seems probable, however, that the greater loss of larvae resulted in large part from alternate wetting and drying of the soil surface. In the unshaded series the yields from the dry and dampened units were extremely, and about equally, low. It was considered probable that the alternate wetting and drying was an important factor in the rapid dying off of the larvae.

The procedures followed in these experiments present two difficulties in the interpretation of results. The extreme reduction of larvae in the first week could have been only in part due to mortality because an unknown percentage of the loss was due to inefficiency of the isolation technic. Also, Augustine's "constantly moist" series, in which dampness was maintained by adding a small amount of water each day, should be in fact regarded as alternately moist and dry. It now has become evident that larvae quickly migrate to the surface of dampened soil and that they are killed by desiccation long before the soil has lost its moist appearance. This factor likewise was inadequately considered in designing the present experiment. It was not anticipated that within a period of less than 30 minutes changes in moisture could produce such critical differences in the migration and survival of larvae. The continuous addition of enough moisture to have delayed desiccation and thus to have permitted migration of larvae as long in the unsampled as in the sampled (pad covered) units very probably would have resulted in significantly more rapid reduction of larvae in the wet series.

The choice of the pad technic of isolating larvae was based on earlier observations in which it was found that yields from it were consistently as good at least as those obtained by the Baermann technic. The special advantage in the pads for the present study, however, was that larvae could be recovered without disturbing the cultures and only those larvae that migrated to the surface at a given

time were removed. Additional advantages were that the apparatus was compact and inexpensive, and the collections of larvae contained relatively few free-living nematodes.

In the coastal plain of the southeastern United States, where in spite of vigorous control efforts hookworm disease is a major public health problem, overall conditions obviously favor the survival of *Necator americanus* as a species. The yield of 46,000 larvae six to eight days after inoculation with 50,000 eggs strongly supports the general view that the open sandy soils of this region provide ideal conditions for the development and rapid migration of the infective stage larvae. On the other hand, findings as regards moisture relations seem somewhat paradoxical. Larvae are quickly killed by desiccation and for that reason, especially in soils which retain moisture poorly, additions of moisture are required for their survival. At the same time, addition of enough moisture to wet the soil results in death of the larvae because it permits their migration to the surface where they tend to remain and perish rather than return to the moist deeper layers. For this reason, the rate at which soil infectivity is reduced varies directly with the frequency of the wettings, provided the intervals between wettings are long enough to allow a certain amount of drying at the surface. In well drained open sandy soils this period may be only a matter of minutes. In the study area, the warm season rainfall is largely limited to a four to six weeks' midsummer period of frequent, short, day-time showers (Beaver 1952). With any given amount of soil pollution by infected individuals, these conditions would seem to produce a very high degree of infectivity of the soil, but for very short periods which, except in midsummer, occur infrequently.

During the summer in southern Georgia almost all of the human stools deposited in and around dooryards are disposed of by coprophagous beetles (Beaver 1952). Cort and Payne (1922) observed a similar situation in Trinidad. Miller (1952) found that beetles sometimes bury entire stools in less than two hours and that three feces-baited traps in a dooryard-sized area attracted enough beetles to have disposed of 8–10 stools daily. According to Payne (1923), infective hookworm larvae may develop from stools buried as deep as 36 inches in sandy loam. Since beetles bury portions of human feces at various depths and in various amounts, they doubtless exert some influence, positive or negative, on the development and persistence of hookworm larvae in soil.

SUMMARY

Eighteen enclosed units of soil were inoculated with equal numbers of hookworm eggs and dampened daily until larvae reached the infective stage. Half were then dampened daily and half left to dry until their turns came to be sampled. Sampling, which began on the 5th day in the wet series and on the 9th day in the dry, was progressive and cumulative, a new unit being added each day until all were under examination. Reduction in numbers in the wet series proved to be significantly more rapid than in the dry series, and on the five days when pairs of wet and dry cultures were sampled simultaneously, the dry cultures yielded 3 to 7 times as many larvae as the wet. When suspensions of in-

fective larvae were poured onto damp soil, less than half of the larvae reached a depth sufficient to escape desiccation.

The results substantiate the view that alternate wetting and drying of soil hastens the death of infective hookworm larvae. Thus, under usual conditions in well drained sandy regions, infective larvae remain viable on the surface for only a few minutes after rain ceases and in the absence of added contamination the soil becomes practically noninfective after brief periods of frequent rainfall.

REFERENCES

AUGUSTINE, D. L. 1922, Investigations on the control of hookworm disease. IX. On the position of the infective hookworm larvae in the soil, *Am. J. Hyg.* **2:** 172–176.

AUGUSTINE, D. L. 1923a, Investigations on the control of hookworm disease. XVI. Variation in length of life of hookworm larvae from the stools of different individuals, *Am. J. Hyg.* **3:** 127–136.

AUGUSTINE, D. L. 1923b, Investigations on the control of hookworm disease. XXII. Further observations on the migrations and the position of infective hookworm larvae in soils, *Am. J. Hyg.* **3:** 416–419.

AUGUSTINE, D. L. 1923c, Investigations on the control of hookworm disease. XXIII. Experiments on the factors determining the length of life of infective hookworm larvae, *Am. J. Hyg.* **3:** 420–443.

BEAVER, P. C. 1952, Observations on the epidemiology of ascariasis in a region of high hookworm endemicity, *J. Parasitol.* **38:** 445–453.

CORT, W. W. 1925a, Investigations on the control of hookworm disease. XXXIV. General summary of results, *Am. J. Hyg.* **5:** 49–89.

CORT, W. W. 1925b, Notes on the effect of burial on infective hookworm larvae, *J. Parasitol.* **12:** 33–38.

CORT, W. W. AND PAYNE, G. 1922, Investigations on the control of hookworm disease. VI. A study of the effect of hookworm control measures on soil pollution and infestation in a sugar estate, *Am. J. Hyg.* **2:** 107–148.

CORT, W. W., ACKERT, J. E., AUGUSTINE, D. L., AND PAYNE, F. K. 1922, Investigations on the control of hookworm disease. II. The description of an apparatus for isolating infective hookworm larvae from soil, *Am. J. Hyg.* **2:** 1–16.

MILLER, A. 1952, Dung beetles (Coleoptera, Scarabeidae) in relation to human feces in a hookworm area of southern Georgia. To be published.

PAYNE, F. K. 1923, Investigations on the control of hookworm disease. XIV. Field experiments on vertical migration of hookworm larvae. (Preliminary report.), *Am. J. Hyg.* **3:** 46–58.

THE GROWTH STIMULATING EFFECT OF APF, TERRAMYCIN HYDROCHLORIDE, VITAMIN B_{12} AND AN UNDETERMINED FACTOR "X" UPON *AEDES AEGYPTI* (L)(DIPTERA, CULICIDAE)

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In many fields of entomological investigation, particularly in the study of insect physiology, toxicology and disease transmission, it becomes necessary to dissect or section insects for study. At such times it is to the advantage of the investigator to have at his disposal insect specimens which are as large as the genetic range of the species will permit and to be able to rear these specimens in the shortest possible period of time.

The growth stimulating effect of antibiotic feed supplements in the ration of chicks and hogs is well established. Different antibiotics are in general dissimilar as to their physical constants, specificity towards micro-organisms and known stability; therefore, different results may be possible with different antibiotics (Matterson and Singsen, 1951; Brown and Luther, 1950). As a consequence, results reported here should not be construed as being representative of results for all antibiotics.

The nutrition of mosquito larvae has been studied by many investigators (Buddington, 1941; Goldberg, *et al.*, 1944; Hinman, 1932, 1933; Rozeboom, 1935; Rouband and Grenier, 1941; Subbarow and Trager, 1940; Trager, 1935a, 1935b, 1942), but the influence of an antibiotic supplement, to my knowledge, has not been reported. Data obtained with chicks is suggestive that the better the quality of the ration with respect to protein and vitamins, the less growth response is obtained by supplementing rations with antibiotics. There was no reason to believe that it should be otherwise with mosquitoes. Well balanced diets of dog or rat chow, liver and yeast preparations, bread, oats, dehydrated blood serum, dehydrated skimmed milk, protozoan cultures and many other substances have been used as larval mosquito food. The problem becomes one of studying the effects of supplementing a well balanced and nutritious ration in an effort to obtain mosquitoes of maximum size in the shortest possible period of time.

EXPERIMENTAL PROCEDURE

A liter of distilled water was placed in each of several white enamel pans (10″ x 8″ x 2″). The rations to be tested were determined, weighed or otherwise prepared in advance. To each pan was added a day's supply of test food (175 mg.). The pans of water and food were placed in an incubator, set at 27°C. overnight. The following day *A. aegypti* eggs were placed in distilled water at room temperature and then incubated at 27°C. to hatch. Upon hatching, 100 larvae were pipetted into each of the rearing pans in which the food had been

standing over night. These pans were then returned to the incubator. A predetermined amount of food was added to each pan daily until the first pupa appeared. Ten fourth-instar larvae were selected from each pan at random and the distance from the anterior margin of the thorax to the base of the siphon of each was measured with an ocular micrometer. An average for each group of 10 was determined. It was calculated that a difference of 0.7 mm. in these averages was statistically significant for this small sample. A Beckman glass electrode pH meter was used to determine the pH of the water in the rearing pans before the larvae were placed into them and again when they were removed. Records were kept on the condition of the water, number of dead larvae and pupae and the length of time to the earliest pupation. Although 27°C. is the usual temperature employed for rearing *A. aegypti*, some tests were conducted at both 27° and 37°C. since an incubator set at the latter temperature was available.

RATIONS EMPLOYED

To reduce the number of mathematical combinations of food, only ground mouse pellets (Rockland Mouse Diet, a product of Rockland Farms, New City, New York—mineral and vitamin contents given on each bag), Bacto-Liver, brewer's yeast (Anheuser and Busch), APF (Animal Protein Factor, Bi-Con 3 + vitamin B_{12} supplement—containing streptomycin and terramycin fermentation solubles), terramycin hydrochloride, and crystalline vitamin B_{12} (saline solution) were utilized. Each of these substances was tested alone and 176 combinations were tested and compared. A standard or control was necessary with which to compare the tests. Since the ground mouse diet alone produced larvae the size of which compared favorably with the largest larval forms reported in nature (Matheson, 1944—gives the natural size of *A. aegypti* to be 8–9 mm.), this food was accepted as the control.

RESULTS

Effect of temperature. A difference of statistical significance appeared between all larvae reared at 27° and 37°C. irrespective of food. Ground mouse food is a typical example. When a pan of 100 larvae were fed 175 mg./day and maintained at 27°C., the average size from the anterior margin of the thorax to the base of the siphon was 7.66 mm. while those maintained on the same food at 37° averaged 5.27 mm. However, the type of food in conjunction with the temperature determined the length of larval duration. In many instances pupation occurred a day earlier at 37°, but as a rule an extra day was spent in the pupal stage offsetting the day gained during larval development. Mortality was considerably higher at 37° than at 27°C., particularly when yeast constituted a portion of the diet.

Effect of diet. When the foods were tested individually at 27°, there was no significant difference in size between those fed ground mouse diet, APF, yeast, and liver, although the first of these produced the largest larvae. All larvae fed

only terramycin hydrochloride or vitamin B_{12} died. Those fed the former were all dead within four days while those fed the latter survived for 65 days. Some larvae survived for 68 days in a control pan to which no food at all was added.

It became evident that better results could be obtained when liver and yeast *per se* were omitted from the diet, and the best results were achieved when ground mouse food was supplemented with APF, terramycin hydrochloride and vitamin B_{12}. Many food combinations were statistically inferior to ground mouse food; others gave size results similar to this control diet. No food combination produced larvae which were significantly larger, statistically, than those produced by the control diet in any given test run; however, several diets produced larvae which were always larger than the controls in all repeat test runs. When data from these repeat runs were analyzed statistically, utilizing all information, it was found that these test diets were significantly superior statistically to the control diet. The larvae feeding on these diets were also significantly larger than those found in nature. The food combination producing the largest larvae was:

a. ground mouse food—50 parts by wt.
b. APF —50 parts by wt. } 175 mg. daily
c. 2.5 mg. terramycin hydrochloride, daily
d. 1.5 ugr. vitamin B_{12}, daily

This diet was run seven times with a control each time (Table 1). It was also repeated decreasing and increasing the amounts of each substance. The addition of only 0.75 ugr. of vitamin B_{12} gave very similar results. Other combinations of this diet were always slightly inferior although not significantly so. Since this was the best of the diets tested, it will be referred to as the S (superior) diet.

Mosquitoes fed both the S diet and the control diet spent less than 5 days in the larval stage and required less than 7 days to develop to adults; mortality was negligible in each (0 per cent in the control diet, 0.28 per cent on the S diet); the water in the pans containing the terramycin was always clear, although a brown precipitate was formed. The size of the larvae produced by the S diet averaged nearly 4 per cent larger than the average of the controls (7.813 mm. to 7.539 mm.). The maximum average size obtained from a pan to which the S diet was added was 8.24 mm., which is better than 9 per cent larger than the average size of those fed the control diet. The largest individual specimen measured 8.86 mm. Its overall length from the anterior margin of the head to the end of the 9th abdominal segment was 10.7 mm., better than 13 per cent larger than the average of the controls. These results are comparable to those obtained with chicks and hogs fed well balanced rations supplemented with antibiotics.

Before placing the larvae in the rearing pans, the pH was always found to be on the acid side, usually ranging from 6.0 to 6.7. There were occasional acid readings of from 5.3 to 5.7. When the larvae were removed, after developing,

the water was always basic with pH readings of from 7.02 to 7.3 among the pans to which the control diet had been added and from 7.4 to 7.87 in the pans to which the S diet was added.

Effect of factor "X". Those diets which proved superior to ground mouse food were repeated a number of times along with controls. Not infrequently the larvae in a repeat run in both the control pans and the test pans would be significantly smaller, statistically, than in previous runs utilizing the same food (Table 1, tests 3 and 4). However, in any given run the larvae in all pans, irrespective of food, would fluctuate up or down in size together. Although the difference in size between two runs utilizing the same food might be significantly different, the difference between the size of the larvae on a control diet and those larvae on the test diet of the same run would be nearly the same as the size difference between the larvae on the control diet and on the test diet of

TABLE 1

Average size of A. aegypti *larvae fed control and S diets in repeated tests*

TEST	ON S DIET	ON CONTROL DIET	DIFFERENCE (S DIET—CONTROL DIET)
1	7.874 mm.	7.506 mm.	+ 0.368 mm.
2	7.841	7.559	+ 0.282
3	8.240	8.175	+ 0.065
4	7.495	7.301	+ 0.194
5	7.587	7.310	+ 0.277
6	7.850	7.429	+ 0.421
7	7.808	7.495	+ 0.323
Average	7.813	7.539	+ 0.276

S.E. of av. diff. = .049

the second run. Among seven test runs of the S diet, the variation between the largest and smallest average size was 0.745 mm. (Table 1). Other diets and controls would sometimes have greater variations. It can therefore be said that under the conditions of the experiment there existed a factor or a series of factors which perhaps played a more influential part in determining size than did controlled diet, temperature, surface volume ration or pH. Because of existing circumstances and conditions, this "X" factor probably cannot be determined in this laboratory. When this "X" factor was working in favor of maximum size, it produced larvae nearly 6 per cent larger than the average of those fed the S diet and better than 9 per cent larger than the average of those fed the control diet. If this factor were known and could be controlled to operate in favor of maximum size at all times, it would theoretically be possible to produce larvae which would average about 5 per cent larger than the average reported here.

DISCUSSION

Although this experiment indicates that a statistically significant increase in the size of *A. aegypti* larvae can be obtained by supplementing an already nutritious larval diet with APF, terramycin hydrochloride, and vitamin B_{12}, it should be kept in mind that growth stimulating effects of different antibiotics may be quite different and it may, therefore, be possible to surpass the size obtained here by using some other antibiotic. Further, the ability of thymidine, desoxyribosides of hypoxanthine, adenine, cytosine and desoxyribosenucleic acid (DNA) to replace vitamin B_{12} in the nutrition of *Lactobacillus leichmanii* 4797 has been established. The activity ratio of thymidine to vitamin B_{12} in the test tube is about 10,000 to 1 and that of DNA to B_{12}, 800,000 to 1 (Wright, L. D. *et al.*, 1951). It is quite possible that one of these substances might prove to be more beneficial than B_{12} in the growth of mosquito larvae.

Since all larvae were hatched from eggs with an unknown history (of age, condition of storing, etc.) and since many batches of eggs were used, it is possible that the "X" factor which determines size and created the significant difference in size between two tests of the same food, can be attributed to the micro-condition of the eggs, or possibly to difference in air pressure at the different times the tests were run or perhaps the season of the year might play a part. The possibility of detergent clinging to washed rearing pans and affecting the size of larvae was ruled out after this was studied and controlled.

The relatively few numbers of larvae per pan may well have contributed to the reduction in the time required for larval development over the times usually reported.

SUMMARY AND CONCLUSIONS

An experiment to determine the possible growth stimulating effects of APF, terramycin hydrochloride and vitamin B_{12} upon *Aedes aegypti* was conducted. It was found that the accepted temperature of 27°C. for rearing these mosquitoes is superior to 37° since the mortality at the latter temperature was too great and the length of time required to attain the adult stage was not reduced.

The average larval size obtained per pan of 100 larvae with daily feedings of the S (Superior) diet, composed of 175 mg. of equal parts by weight of ground mouse food, and APF, 2.5 mg. of terramycin hydrochloride, 1.5 ugr. of vitamin B_{12}, was about 4 per cent larger than the largest reported in nature and those fed the well balanced control diet of 175 mg. ground mouse food daily.

A size variation of statistical significance occurred between larvae in different test runs utilizing the same diet. This indicated that there was some unknown and uncontrolled factor, factor "X", which was as influential in determining size as the controlled factors of diet, temperature, pH, and water surface ration. When this functioned in favor of maximum growth, it produced an almost 10 per cent increase in size over the average size of the controls.

Mosquitoes fed the S diet averaged a fraction of a day longer in the larval

stage than did those on the control diet (4.7 against 4.5 days) but both reached the adult stage in less than 7 days.

It is, therefore, possible to rear *A. aegypti* in the laboratory which are significantly larger statistically than those in nature and those fed a well balanced control diet; however, the average maximum size as controlled by the genetics of the species has not as yet been attained. This might be approached by the determination and favorable utilization of factor "X". Other antibiotics and substances such as DNA substituted for vitamin B_{12} might also bring about an average size increase superior to that reported here.

ACKNOWLEDGMENT

Thanks are due to the International Health Division of the Rockefeller Foundation for the mosquito eggs; to Chas Pfizer and Co., Inc. for the APF and terramycin hydrochloride; to Merck and Co., Inc. for the crystalline vitamin B_{12}; and to Dr. John Fertig, Professor of Biostatistics, School of Public Health, Columbia University, for the statistical analysis of the data.

REFERENCES

Brown, J. H., and Luther, H. G. 1950, Effect of antibiotics and growth stimulating substances in the ration of growing and fattening hogs, *J. Animal Sci.* **9:** 650.

Buddington, A. 1941, The nutrition of mosquito larvae, *J. Econ. Ent.* **34:** 257–281.

Goldberg, L. *et al.* 1944, Relation of "folic acid" to the nutritional requirements of the mosquito larva, *Nature* **154:** 608–610.

Hinman, E. H. 1932, The role of solutes and colloids in the nutrition of anopheline larvae, *Am. J. Trop. Med.* **12:** 263.

——— 1933, The role of bacteria in the nutrition of mosquito larvae. The growth-stimulating factor, *Amer. J. Hyg.* **18:** 224–246.

Matheson, R. 1944, The mosquitoes of North America. Comstock Publishing Co., Inc.

Matterson, L. D., and Singsen, E. P. 1951, A comparison of several antibiotics as growth stimulants in practical chick-starting rations. Bull. 275, Storrs Agricutural Experiment Station, College of Agriculture, Univ. of Conn., Storrs, Conn.

Roubaud, E., and Grenier, P. 1941, Some observations on the food of mosquito larvae (B factors and vitamins), *Bull. Soc. Path. Exot.* **35:** 215–219.

Rozeboom, L. E. 1935, The relation of bacteria and bacterial filtrates to the development of mosquito larvae, *Am. J. Hyg.* **21:** 167–179.

Subbarow, Y., and Trager, W. 1940, The chemical nature of growth factors required by mosquito larvae. II. Pantothenic acid and vitamin B_6, *J. Gen. Physiol.* **23:** 561–568.

Trager, W. 1935a, The culture of mosquito larvae free from living microorganisms, *Amer. J. Hyg.* **22:** 18–25.

——— 1935b, On the nutritional requirements of mosquito larvae (*Aedes aegypti*), *Am. J. Hyg.* **22:** 475–493.

——— 1942, The chemical nature of growth factors required by mosquito larvae, *Proc. N. J. Mosq. Exter. Assoc.* **29:** 46–48.

Wright, L. D. *et al.* 1951, Microbiological methods in vitamin research, Vitamin Methods. Vol. II. Edited by Paul György, Academic Press, Inc., N. Y.

STINGRAY ATTACKS AND THEIR TREATMENT[1]

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The selachian order Batoidea contains the two stingray families: Dasyatidae, the stingrays proper (Figs. 1, 3, 4); and Myliobatidae, the eaglerays or bat-rays (Fig. 2). These two families comprise some of the most venomous fishes known to man. Stingrays are common inhabitants of all tropical and most temperate seas. Certain species, e.g., members of the dasyatid subfamily Paratrygoninae live in freshwater streams, but most stingrays are marine forms. Activities such as beach bathing, wading, seining, fishing and underwater demolition work take man into the environs of the stingray. The reports of stingray attacks in the literature and testimonials of victims attest to the frequency in which man departs from the stingray habitat "sadder but wiser" for his experience. Frequent requests for information regarding stingray attacks and the treatment of wounds produced by these fishes have served as stimuli for the present writing.

HABITS OF STINGRAYS

Many stingray attacks might have been prevented if the victim had known the habits of the fish and been more careful. Stingrays are most likely to be encountered in the warm waters of shallow bays, estuaries and sloughs. Most species have the habit of lying completely or partially buried in the upper layer of a muddy bottom. Eaglerays seem to prefer resting on top of the mud or sand and may be frequently observed swimming at the mid-water level. Attacks generally occur as a result of an unwary victim stepping on a partially buried stingray. The fish is then in a position to thrust the sting into the flesh of the victim. A shuffling gait frightens the fish from cover and thus lessens the danger of stepping on it. In wading in shallow, muddy bays a person should shuffle his feet through the mud.

STINGING ACTION

Ancient writings abound in superstition and fantasy as to the stinging prowess of rays and their venomousness. The following excerpt from Pliny (cited by Gudger 1943) is typical: ". . . there is nothing that is more to be dreaded than the sting which protrudes from the tail of the Trygon, by our people known as the Pastinaca, a weapon five inches in length. Fixing this in the root of a tree, the fish is able to kill it; it can pierce armour too just as though with an arrow, and to the strength of iron it adds all the corrosive qualities of poison." More recent writers, Yarrell (1859), Coupin (1889), Hilzheimer (1913), Ronka and

[1] This investigation was supported by a research grant from the National Institutes of Health, Public Health Service, and a contract with the Office of Naval Research, Department of the Navy (NONR-205(00)).

Roe (1945), and others, claim that when stingrays are excited they will aggressively wrap their long tails around the victim and thrust their sting into the

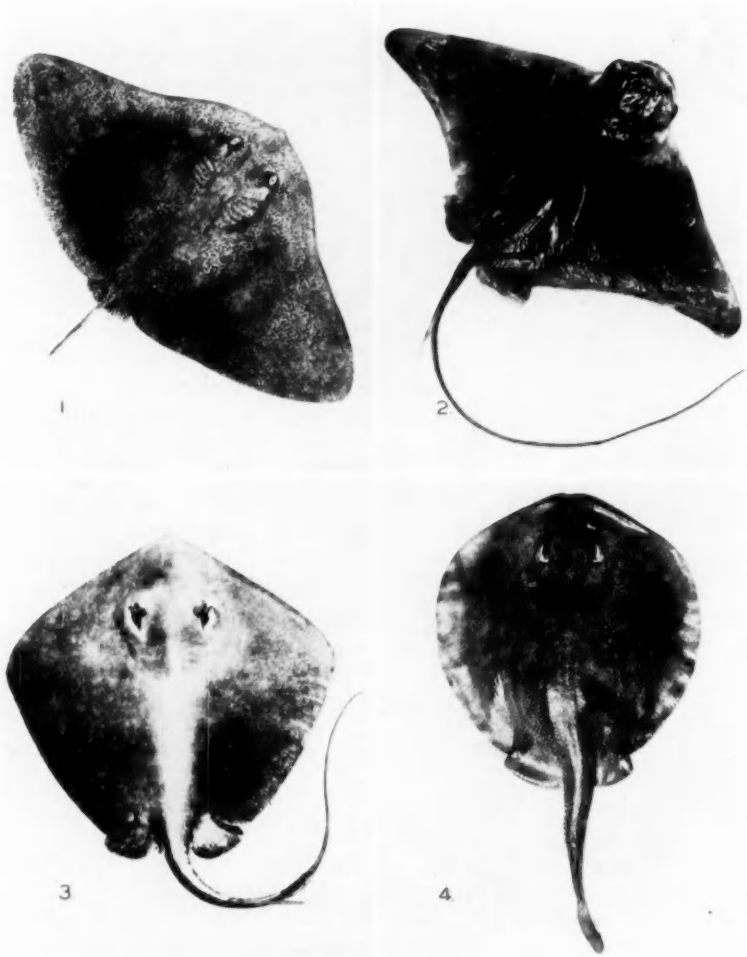


FIG. 1. *Gymnura marmorata* (Cooper), the California butterflyray. (From Kumada)
FIG. 2. *Holorhinus californicus* (Gill), the bat-ray.
FIG. 3. *Dasyatis dipterura* (Jordan & Gilbert), the diamond stingray. (From Garman)
FIG. 4. *Urobatis halleri* (Cooper), the round stingray. Showing representative North American species of the four stingray types.

flesh. There is no scientific evidence to support the idea that the whip-like tail of the stingray is used as a prehensile organ. Furthermore, the anatomy of the caudal appendage is such as to preclude its use for grasping. Undoubtedly the

long tail of these fishes may be wrapped around the victim, but it is a passive rather than an active measure. Judging from histological sections and field observations the whip-like caudal appendage plays a significant role as a sensory organ.

Campbell (1951) has published the only critical analysis of the stinging action of stingrays that has been found to date. "Though this fish [speaking of *Urobatis halleri* (Cooper), the round stingray] is greatly feared by bathers, in the aquarium it proved very reluctant to sting." He found that in order to evoke the stinging response it was necessary to pin the fish to the bottom of the tank by the sudden application of a large rubber suction disc on the end of a stick. This technique produced repeated thrusts with the sting. In rays in which the spinal cord had been severed, the stinging reaction followed stimulation of the caudal one-third of the pectoral fins and the entire surface of the pelvic fins. When the trunk was stimulated there was no response. His experiments revealed that stinging was always directed to the side by a bending of the tail toward the evoking stimulus. The tip of the tail takes no active part in the stinging action.

The junior author recently participated in a seining operation in which more than 170 stingrays were captured. About 160 of these rays were *Urobatis halleri*. The abundance of specimens afforded an excellent opportunity to make field observations on the stinging actions of these fish. The stimulus consisted of poking the back of the fish with a blunt stick. It was noted that for a quick stinging response the stimulus had to be applied to the posterior third of the ray's body. The stinging reaction time rapidly decreased as the application of stimuli progressed in the anterior direction. The stinging response in the series of rays tested was found to be consistent but varied with the type of stimulus used. When a stick was applied to the posterior third of the back, the ray immediately arched the tail through a vertical plane over its back and struck with precision at the probe. There were no horizontal movements or general lashing of the caudal appendage, but a very precise thrust. When a more diffuse stimulus was applied to the posterior third of the ray's body, both vertical and horizontal movements were observed. At a later date a second series of urobatids were captured and it was noted that both the vertical and the horizontal type of thrust as observed by Campbell were used.

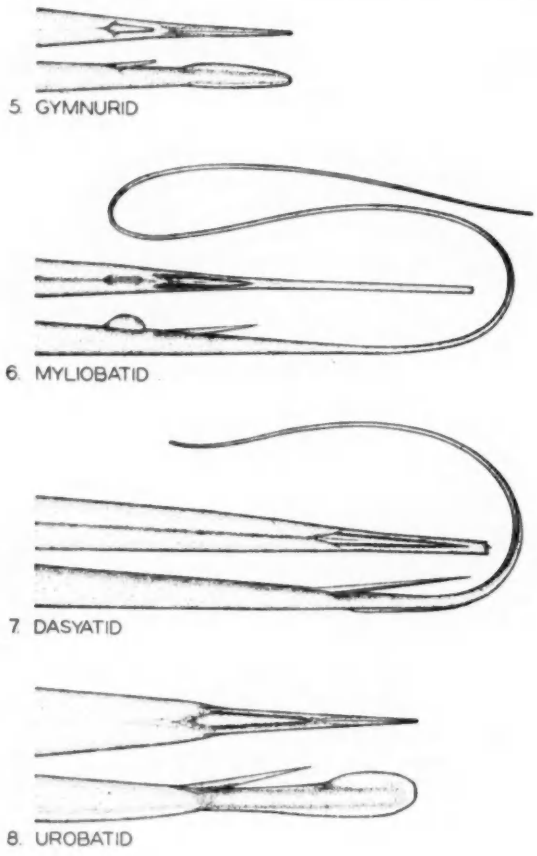
TYPES OF STINGRAY CAUDAL APPENDAGES

The venom apparatus or sting of stingrays is an integral part of the caudal appendage. The caudal appendage of stingrays can be divided into four general anatomical types according to their morphological adaptability as defensive organs.

1. *Gymnurid type* (Fig. 5): Caudal appendage cylindrical and tapering in cross section, greatly reduced in size. Sting small, about 2.5 cm. in length, usually placed in middle or proximal third of tail. Dorsal and caudal fins variable, present as cutaneous folds or indistinct. Cuneiform area usually moderately developed. Example: *Gymnura*, the butterfly rays.

2. *Myliobatid type* (Fig. 6): Caudal appendage cylindrical and tapering in cross section, but terminating in a long whip-like tail. Vertical caudal fin fold

absent or indistinct. A single dorsal fin present immediately proximal to origin of sting. Sting generally located on proximal portion of basal third of tail, moderate to large in size, usually about 5 to 12.5 cm. in length, cuneiform area



FIGS. 5-8. Showing the various types of caudal appendages of stingrays, arranged in their order of adaptability as a defensive weapon.

moderately developed. Examples: *Holorhinus*, *Myliobatis*, *Aëtobatis*, *Rhinoptera*, the eaglerays or bat-rays.

3. *Dasyatid type* (Fig. 7): Caudal appendage depressed proximal to sting, becoming cylindrical and tapering in cross section distally and finally terminating in a long whip-like tail. Dorsal fin absent. Sting, moderate to large, may attain a length of 37.5 cm. in some species, usually located in the distal portion

of the basal or middle third of tail. Vertical caudal fin fold indistinct in some species but pronounced in others. Cuneiform area usually poorly developed. Examples: *Dasyatis*, the true stingrays; *Paratrygon*, the South American freshwater stingrays.

4. *Urobatid type* (Fig. 8): Caudal appendage relatively short, muscular, depressed proximal to sting, not produced as a whip-like structure, becoming compressed distal to sting and usually forming a more or less distinct caudal fin. Dorsal fin present or absent. Bodies of lateral caudal muscle fasciculi diminish abruptly in size as they approach the origin of the sting and continue distally as tendinous attachments. Sting usually located in middle or distal third of tail, moderate in size, usually about 4 cm. in length, cuneiform area moderately to well developed, forming a distinct depression in *Urobatis halleri*. Example: *Urobatis*, the round stingrays.

The gymnurid type with its short tail, small proximally located sting and minimal caudal musculature is the least suited as a defensive weapon. The stinging apparatus is most highly developed in the urobatid stingrays in which the caudal appendage is a powerful muscular organ with a well-developed cuneiform area and distally placed sting. The myliobatid and dasyatid types are intermediate forms. In regions where the four types of stingrays are present, it will be noted that the majority of attacks reported have been produced by stingrays possessing the urobatid and dasyatid type of caudal appendage. While the relative abundance and habits of the various species of stingrays are additional factors which must be taken into consideration, the significance of the morphological adaptation of the venom apparatus is not to be minimized. This classification of caudal appendage types is an arbitrary one based upon the adaptability of the structure as a defense organ.

THE STINGRAY VENOM APPARATUS

The question whether stingrays possess a true venom apparatus has been debated by many writers. Excellent historical summaries of the subject have been written by Evans (1916, 1923) and Gudger (1943). Antonio Porta (1905) and H. Muir Evans (*ibid.*) were probably the first to describe the microscopic anatomy of the venom apparatus of the two European stingrays, *Dasyatis pastinaca* (Linnaeus) and *D. violacea* (Bonaparte). Halstead and Modglin (1950) have described the venom apparatus of *Holorhinus californicus* (Gill), the California bat-ray. Ocampo, Halstead and Modglin (in press) have reported on the microscopic anatomy of *Aëtobatus narinari* (Euphrasen), the spotted eagleray. There is now histological evidence which indicates that the stingray species studied thus far do possess a true venom apparatus.

ANATOMY[2]

The venom apparatus of stingrays consists essentially of the serrate spine (Fig. 9, 11) and its enveloping integumentary sheath. The terms sting and venom

[2] The authors have followed the anatomical terminology as suggested by Ocampo, *et al.* (in press).

apparatus are synonymous. The term spine refers to the serrate "bony" (vasodentinal) structure minus its integumentary sheath. The spine is located on the dorsum of the caudal appendage at a variable distance from the base of the caudal appendage. Stingrays generally possess only a single spine at a time, but it is not unusual to find a specimen with two or more. Apparently the spine

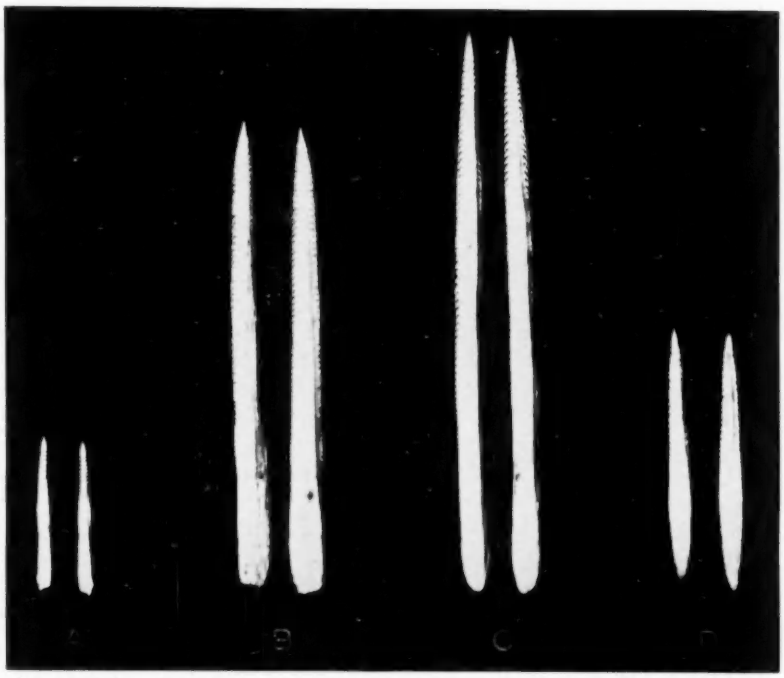


FIG. 9. Spines from four representative stingrays showing comparative morphology. Left spines, dorsal view; right spines, ventral view. A, *Gymnura marmorata*, B, *Hahnichinus californicus*, C, *Dasyatis dipterura*, D, *Urobatis halleri*.

remains until it is removed by injury. There is no evidence to support the idea that they are shed each year. As the young spine grows out from the caudal appendage it takes with it a layer of skin (the integumentary sheath) which continues to insheath the spine until it is removed by trauma or wear. The spine is connected to the caudal musculature by tendinous attachments.

The mature spine is an elongate, tapering vasodentinal structure, bilaterally serrate with a variable number of sharp, recurved teeth and terminates in an acute arrow-shaped tip. The dorsal surface of the spine is marked by a number of irregular, shallow, longitudinal depressions. The serrate edges of the spine are termed the dentate margins (Fig. 10a). Medial to the dentate margins of the spine, on the ventral surface, will be found two longitudinal grooves, one on either side, which are called the ventrolateral-glandular grooves.

Examination of the grooves of an unsheathed or traumatized sting will reveal that they each contain a strip of soft, spongy, grayish tissue extending throughout the length of the groove. This tissue is the remnant of the integumentary sheath. The integumentary sheath is gradually worn away over a period of time so that the only tissue that remains lies in the protection of the grooves.

Microscopic examination of histological cross sections of an intact sting reveals that it is roughly diamond shape and consists of a broad T-shaped dentinal structure completely enveloped by a layer of integument. The integument is comprised of two layers (Figs. 10 a.b.). The inner layer, the dermis, consists of areolar connective tissue and vascular channels. The outer layer, the epidermis, is composed of modified squamous epithelium containing many glandular cells. A cross section of the ventrolateral-glandular groove has been termed the glandular triangle. In the stingray spines studies to date, it was found that the glandular activity is generally most concentrated in the epidermis in the immediate vicinity of the ventrolateral-glandular grooves. The venom is produced as a result of the glandular cells undergoing a holocrine type of lysis. The tissue lying within the ventrolateral-glandular grooves is believed to be the principal site of venom production. There is no histological evidence to indicate that the venom is secreted through a duct.

The thickened wedge-shaped portion of the integument on the dorsum of the caudal appendage ventral to the sting is known as the cuneiform area (Fig. 11). Toxicological studies of the cuneiform integument indicate that the glandular cells of this area also secrete venom. Thus, the sting would be bathed in mucus and venom originating from the ventrolateral-glandular-groove tissue and the cuneiform area.

EXPERIMENTAL STUDIES ON STINGRAY VENOM

The literature dealing with stingray venom appears to be the product of a single worker, Vellard (1931, 1932) and deals with the South American freshwater stingray, *Paratrygon motoro* (Müller and Henle).[3] Vellard obtained crude extracts by scraping the ventrolateral-glandular grooves of the sting with a scalpel and triturating the material in both distilled water and physiological saline solution. The solution gave an acid reaction with litmus and was highly toxic when injected into animals. Tests were made with crude extracts and by stabbing the animals with intact stings. The venom was tested on dogs, rabbits, mice, pigeons, reptiles and batrachians. Dogs usually survived the crude extract injections, but frequently died when wounded with the intact sting. Intravenous and intramedullary routes of administration were found to be the most efficacious. Vellard found that one-fourth of a sting killed a 30 gm. mouse within 10 to 20 minutes. Boiling, strong acids and alcohol were said to destroy the action of the venom. Solutions of the venom stored at 5°C. were reported to lose their potency within a few days.

[3] The two species *Taeniura dumerilii* and *T. mulleri* as mentioned by Vellard (*ibid.*) are now considered as synonyms of *Paratrygon motoro* (Müller and Henle) according to Garman (1913) and Fowler (1948).

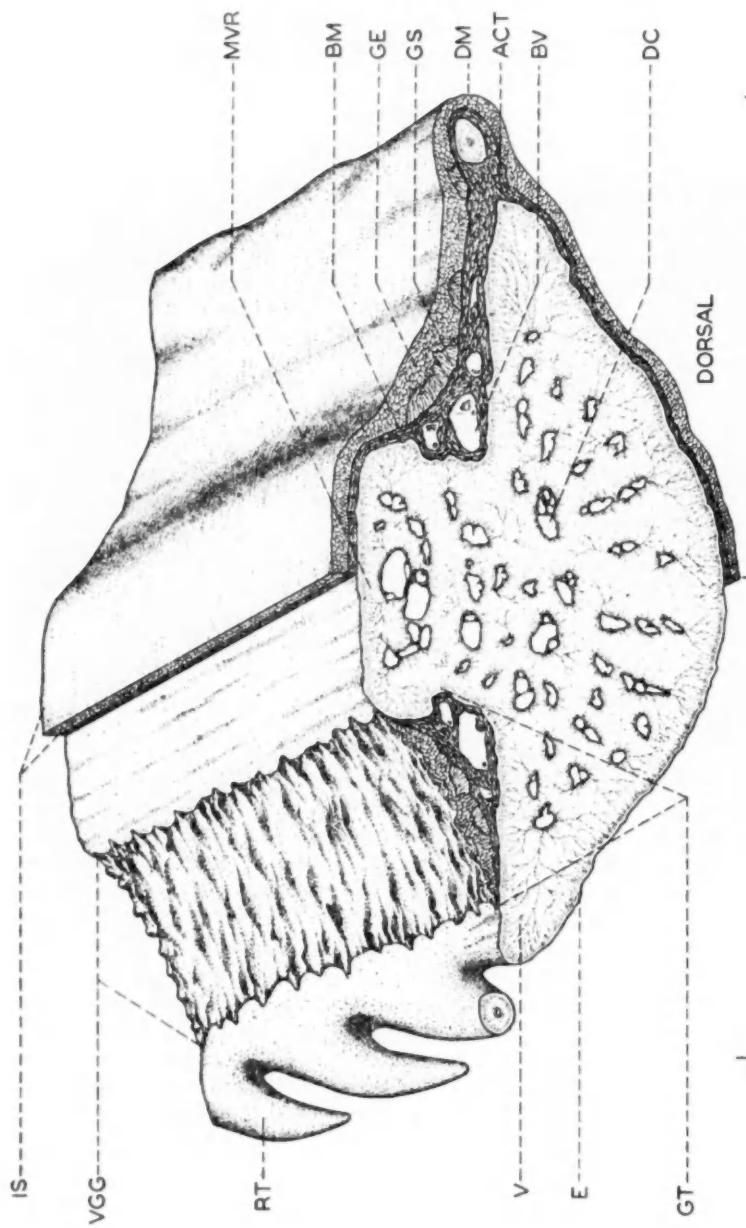

MVR
BM
GE
GS
DM
ACT
BV
DC
DORSAL
INTACT STING
TRAUMATIZED STING
IS
VGG
RT
V
E
GT

FIG. 10a

A series of tests was recently conducted in our laboratory on the venom of *Urobatis halleri*. Distilled water was used as the extractant. One ml. of crude extract contained the venom extracted from the ventrolateral-glandular grooves

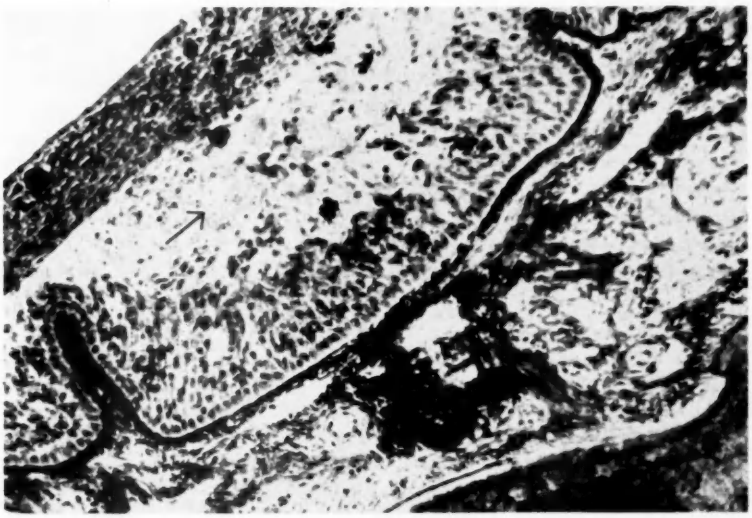


FIG. 10b

FIG. 10a. Schematic drawing of a three dimensional view of a section taken from the middle third of a sting of *Holorhinus californicus* showing the anatomical relationships of a traumatized and intact sting. Cross section prepared with camera lucida (× 100). ACT: areolar connective tissue; BM: basement membrane; BV: blood vessel; DC: dentinal canals; DM: dentate margin; E: enamel; GE: glandular epithelium; GS: glandular secretion (venom); GT: glandular triangle; IS: integumentary sheath; MVR: median ventral ridge; RT: retrorse tooth; V: vasodentine; VGG: ventrolateral glandular groove. b. Photomicrograph of a cross section of a ventrolateral glandular groove of *Aetobatus narinari* (Euphrasen) (× 165). Arrow indicates area of glandular secretion or venom.

FIG. 11. Caudal appendage of a representative stingray showing the gross morphology of the venom apparatus. CA: cuneiform area; IS: integumentary sheath; S: spine.

of two stings. Tests were made by injecting 1.0 ml. of the extract intraperitoneally into four white mice (CC_1 strain) weighing 20.5 gm. each. Their death times varied from 14 minutes to 1 hour and 32 minutes. Extracts were prepared from a series of cuneiform areas and they were also found to be toxic. Extracts

prepared from slime were slightly toxic, but extracts of caudal appendage integument proved to be nontoxic when injected into mice. Details of the investigation will be presented elsewhere at a later date.

CLINICAL CHARACTERISTICS OF STINGRAY WOUNDS

The sharp, arrow-like tip and backward pointing teeth of the stingray spine make the structure an effective weapon. The spine is so constructed that penetration of the victim's flesh is accomplished with ease, but the removal of the barb is difficult and lacerates the tissues as it is withdrawn. The sheath of the sting is easily damaged. When the sting penetrates the flesh the sheath is torn, the dentate margins of the sting are exposed and venom released. Laceration of the tissues aids in the absorption and distribution of the toxin, thus producing a violent tissue reaction.

Stingray injuries are generally of the puncture-wound variety and occur most frequently about the ankle joint and foot since they are usually contracted as a result of stepping on the ray. Liggins (1939), Whitley (1940), and Ronka and Roe (1945) have reported instances in which the wounds occurred in the chest about the heart. Pain is the predominant symptom and usually develops immediately after the wound is produced. Tropical stingrays, especially freshwater species, are said to produce extremely painful wounds (Crevaux and Le Janne 1882, Coupin 1899, Bassler 1942). The pain is variously described as sharp, shooting, spasmodic or throbbing and localized or radiating in character. The area about the wound at first has an ashen appearance, later becoming cyanotic, followed by redness and swelling. Crevaux and Le Janne (*ibid.*) cite a case in which edema extended from the right heel to the epigastrium, the edematous area being cyanotic in appearance. Evans (1945) mentions a case in which the predominant symptoms were painful, shallow respiration and intermittent spasms of the abdominal muscles. In the cases cited by Coupin (*ibid.*) the patients developed convulsions and died. Deaths resulting from stingray attacks have also been reported by Mordecai (1860), Crevaux and Le Janne (*ibid.*), Meinard (1905), Gudger (*ibid.*), Whitley (*ibid.*) and others. Primary shock may be present and manifested by such symptoms as faintness, weakness, nausea, loss of consciousness, cold clammy skin, rapid weak pulse, low blood pressure and respiratory distress. Prior to the advent of the sulfonamides and antibiotics, secondary bacterial infections were a serious complication which frequently resulted in gangrene and severe tissue sloughs.

CASE REPORTS

Case No. 1.—A 24-year-old white male, in good health, was hunting abalone at Miller's landing, Baja California. While wading in shallow water a few hundred feet from shore he accidently stepped upon the back of a partially buried California bat-ray, *Holorhinus californicus*. The fish was estimated to have a pectoral fin spread of about 4 feet. The ray immediately jabbed its sting in the right foot just inferior and posterior to the inner side of the ankle near the bony protuberance. Pain was described as immediate and intense—"felt as

though someone had stuck me with a hot electric wire of high voltage" — and was soon felt throughout his entire leg. As the fish swam away it jerked the sting from the wound. Examination of the wound revealed that it was bleeding profusely. The victim attempted to walk to shore, but found that his wounded leg would not support his weight. Pain began to depart from his leg as a sensation of numbness gradually developed. During this time he was still able to move his toes. He then attempted to hop back to shore on one leg. He went about 20 yards, lost consciousness and collapsed into the water. The victim was rescued by a companion who dragged him to shore. After a few minutes he regained consciousness for a short time and then fainted again. During this time his companion lanced the wound, which had become greatly swollen and cyanotic in appearance, with a razor blade and attempted to encourage bleeding. Shortly after lancing the wound the patient regained consciousness and began to complain of the pain returning which was throbbing and becoming progressively more intense. A few minutes after the incident some Mexicans arrived on the scene. They claimed that the local native treatment was to apply hot cactus to the wound. (Judging from the description given, the cactus was *Lophocereus Schottii* (Engelmann) which grows to about 7 feet in height, with single hexagonal limbs 6 to 7 inches in diameter, sparsely covered with spines.) The cactus was cut into short pieces, heated over an open flame and applied directly to the wound. The treatment resulted in blistering the skin but produced a distinct drawing sensation which extended to the groin. Fresh pieces of heated cactus were placed on the wound about every five minutes and the treatment continued for about one hour. The intensity of the pain had greatly diminished by the time the treatment was completed. However, the wound continued to be sore for the next few days during which time hot soaks were used at periodic intervals. In the meantime cellulitis developed in the area about the wound. On the sixth day the patient visited a local physician who administered 1500 units of tetanus antitoxin and 500,000 units of penicillin and advised him to seek hospitalization at the earliest possible date, since the wound infection was becoming progressively worse.

On October 12, 1952 he was admitted to the Long Beach Naval Hospital. Diagnosis upon admission was lymphangitis and cellulitis accompanied by a severe brawny edema of the lower third of the right leg. The wound was situated in the center of a large vesicle with evidence of a previous excision. Granulation tissue had started to fill the wound. The lymphatics of the medial aspect of the thigh were tender to palpation. Temperature was 99.8° F. The patient appeared mildly toxic, otherwise the physical examination was essentially negative. It was finally necessary to cut away the dead flesh around the wound because of excessive laceration and subsequent necrosis of the tissues. Treatment consisted of 100,000 units of penicillin every four hours for the first twenty-four hours and 100,000 units twice a day thereafter. The penicillin was supplemented by streptomycin, 1.0 gm. initial dose and 1.0 gm. twice a day thereafter. Antibiotic therapy was continued until all signs of infection had subsided. On the second day he received 1.0 cc. of tetanus toxoid as an added precaution. During the course of antibiotic therapy the patient received moist hot packs at frequent intervals.

The packs had to be discontinued on the eighth day because of excessive maceration of the skin in the area about the wound.

The patient was examined by the senior author on October 18, 1952. There was observed an ulcerated, draining wound about 2.5 to 3.5 cm. in diameter. There was no evidence of an active infection, the swelling had subsided and the wound appeared to be healing nicely. Patient stated that he was able to walk on his foot but his toes still tingled and felt numb. Time did not permit further follow-up of the case.

FIG. 12. Stingray wound produced by *Aetobatus narinari*, the spotted eagleray.

Case No. 2—A Mexican fisherman about 20 years of age captured a large adult specimen of the spotted eagleray, *Aetobatus narinari*, about 30 km. north of Vera Cruz, Mexico. The ray was placed in the bottom of his fishing dugout. During the course of fishing activities he slipped and stepped on the sting of the ray. The sting penetrated the heel of his left foot to a depth of about 5 cm. The pain was said to be immediate and intense, causing the victim to cry out in anguish. Removal of the sting was difficult and resulted in additional trauma to the tissues. The leg rapidly became swollen and it was found necessary to take the victim to the local emergency hospital. Apparently the treatment consisted of débridement, penicillin injections and hot soaks. Details of the treatment were not available because of the language barrier. The wound was observed 8 days later by the senior author at which time the wound was found to be clean, healing and the swelling subsided (Fig. 12).

TREATMENT OF STINGRAY WOUNDS

The clinical effects resulting from a stingray attack are the products of mechanical trauma and the secretion of venom. Some stingray species are capable of producing serious wounds which if left untreated can result in death of the patient. First aid promptly and correctly administered will help to attenuate the severity of the attack. Stingrays do not inject large quantities of venom as do venomous snakes, hence the clinical effects are generally more localized. The use of the tourniquet is of questionable value. The wound should be incised, bleeding encouraged and suction applied. Débridement and irrigations of the wound with 0.01 per cent (1:10,000) solution of potassium permanganate are generally advisable. If the wound is large a small drain should be left in the wound for a day or two. The application of hot fomentations and soaking the limb in hot magnesium sulfate (epsom salts) solution have been found efficacious. Infiltration of the wound area with 0.5 to 2 per cent procaine has been used with good results. In severe cases opiates and barbiturates will be required. Since secondary bacterial complications occur in most instances the patient should receive a prophylactic course of antibiotics. In the event of primary shock supportive therapy will be needed, *i.e.*, cardiac stimulants, etc. A course of tetanus antitoxin is an advisable recautionary measure.

SUMMARY

The habits of stingrays, stinging action, types of caudal appendages and the structure of the venom apparatus of stingrays are described and the results of experimental studies on stingray venom, the clinical characteristics of stingray wounds and their treatment are briefly discussed.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to the Translating Unit, National Institutes of Health, Public Health Service for the translations of foreign publications; to Mr. Leonard Kuninobu for the preparation of illustrations; and to the Long Beach Naval Hospital for the use of data given in Case No. 1.

REFERENCES

Bassler, H. 1942, A sting-ray attack on a man on the upper Amazon, *Sci.* **95** (2490): 274.

Campbell, B. 1941, The locomotor behavior of spinal elasmobranchs with an analysis of stinging in *Urobatis*, *Copeia* (4): 277–284.

Coupin, H. 1899, Les poissons dangereux, *Méd. mod.* **10:** 681–684.

Crevaux, J., and Le Janne, E. 1882, Récit du troisieme voyage dans L'Amérique Équatoriale, *Arch. méd. Nav.* **37:** 50–53.

Evans, H. M. 1916, The poison-organ of the sting-ray (*Trygon pastinaca*), *Proc. Zool. Soc., London*, **29:** 431–440.

Evans, H. M. 1923, The defensive spines of fishes, living and fossil, and the glandular structure in connection therewith, with observations on the nature of fish venoms, *Philosoph. Trans. Roy. Soc., London.* (B) **212:** 1–33.

Evans, H. M. 1945, Toxic properties of sting-ray's sting, *British Med. J.* (4413): 165.

Fowler, H. W. 1948, Os peixes de aqua doce do Brasil, *Arq. Zool. São Paulo* **6:** 1–204.

Garman, S. 1913, The plagiostomia (sharks, skates and rays), *Mem. Mus. Comp. Zool.* **36:** 1–515.

GUDGER, E. W. 1943, Is the sting ray's sting poisonous? *Bull. Hist. Med.* **14:** 467–504.

HALSTEAD, B. W., AND MODGLIN, F. R. 1950, A preliminary report on the venom apparatus of the bat-ray, *Holorhinus californicus*, *Copeia* (3): 165–175.

HILZHEIMER, M., AND HAEMPEL, O. 1913, *Handbuch der Biologie der Wirbeltiere*, Verlag Von Ferdinand Enke, Stuttgart, pp. 150–154.

LIGGINS, J. B. 1939, An unusual bathing fatality, *New Zealand Med. J.* **38:** 27–29.

MEINARD, D. 1905, Piqûres de poisson venimeux, *Bull. méd. Algérie* **16:** 357–360.

MORDECAI, E. R. 1860, Wound inflicted by a sting-ray: mortification—amputation—result, *New Orleans Med. News and Hosp. Gaz.* **7:** 679–680.

OCAMPO, R. R., HALSTEAD, B. W., AND MODGLIN, F. R. In press. The microscopic anatomy of the caudal appendage of the spotted eagleray, *Aëtobatus narinari* (Euphrasen) with special reference to the venom apparatus. *Anat. Rec.*

PORTA, A. 1905, Ricerche anatomiche sull'apparecchio velenifero di alcuni pesci, *Anat. Anz.* **26:** 232–247.

RONKA, E. K., AND ROE, W. F. 1945, Cardiac wound caused by the spine of the stingray (Suborder—Masticura), *Milt. Surg.* **97:** 135–137.

VELLARD, J. 1931, Venin des raies (*Taeniura*) du Rio Araguaya (Brésil), *Compt. Rend. Acad. Sci., Paris* **192:** 1279–1281.

VELLARD, J. 1932, Mission scientifique au Goyaz et au Rio Araguaya, *Mém. Soc. Zool. France* **29:** 513–539.

WHITLEY, G. P. 1940, *The fishes of Australia. Part 1, Sharks etc.*, Royal Zool. Soc. New South Wales, pp. 193–225, figs. 222–257.

YARRELL, W. 1859, *A history of British fishes*, John Van Voorst, London **2:** 591–603.

A CLINICAL AND PATHOLOGICAL ANALYSIS OF SNAKE BITES ON THE PANAMA CANAL ZONE

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Snake bites on the Panama Canal Zone are infrequent. This report covers the clinical and pathologic findings of the fifty-five cases of known snake bite recorded at Gorgas Hospital during a twenty-seven year period (1925–1951). The victims were residents of the Canal Zone or immediate vicinity. Autopsies were performed on the seven fatal cases.

SPECIES OF SNAKES ENCOUNTERED

Only nineteen of the fifty-five cases offered snakes for identification, eighteen of which were representative of the important poisonous snakes of Panama, as described by Clark (1942) (Chart 1).

Six of the cases bitten by snakes positively identified as poisonous developed no evidence of venom poisoning (Chart 2). It should be pointed out that the lack of venom poisoning in no way means the offending snake was not venomous. The toxicity and dosage of the venom injected is of primary importance. Factors that influence the toxicity of snake venom include (a) how recently food has been killed and eaten, since following a meal the venom is very dilute, (b) the type and size of the species and (c) the manner and location of fang penetration. Without doubt, venom varies both in quantity and toxicity in the same snake from time to time (Schöttler, 1951).

The chart on the tabulation of identified snakes reveals that the most important poisonous snake in Panama is the *fer de lance*. In other regions of Central and South America, members of the *Bothrops* genus are also among the most important venomous snakes. It has been said by Clark (1942) that eighty to eighty-five per cent of the poisonous snake bites in Panama are by the Bothropic group. Over fifty per cent of the identified snakes in this series were *B. atrox*, and analysis of the symptomatology produced in the cases of venom poisoning by the unidentified snakes would lead one to believe that Clark's percentile figure, if anything, is on the conservative side.

CLINICAL ANALYSIS OF SNAKE BITE CASES

All fifty-five cases were seen by members of the hospital staff, and records are available. The cases have been divided into two groups (Chart 3) solely on the basis of the clinical picture presented by the patient. Initially no attempt was made to correlate clinical symptomatology with identified species. Group A, thirty-two cases, constitutes those with no evidence of venom poisoning and Group B, twenty-three cases, those with definite venom effects.

It has been necessary to reject the single element of swelling as evidence of venom poisoning. Many of the cases which were doubtless nonpoisonous snake

bites had rather severe swelling from the improper use of the tourniquet alone. Likewise, six of the bites known to have been inflicted by poisonous snakes showed only swelling, which also could be attributed to a tourniquet applied with a little too much zest. Instances are known of bites by boa constrictors in which no tourniquet was applied but which were followed by severe swelling and edema from trauma alone (Johnson, 1952).

CHART 1

Tabulation of identified snakes

1. Fer de lance, *Bothrops atrox*	10
2. Hog nosed viper, *Bothrops lansbergii*	3
3. Palm viper, *Bothrops schlegelii*	2
4. Coral, *Micrurus*	2
5. Bushmaster, *Lachesis muta*	1
6. Boa constrictor	1

CHART 2

Distribution as pertaining to clinical symptomatology

19 cases with snakes identified
- 1—non-poisonous
- 18—poisonous
  - 6—no evidence of venom poisoning
  - 12—evidence of venom poisoning

36 cases snakes not identified
- 25—no evidence of venom poisoning
- 11—evidence of venom poisoning

CHART 3

Classification of presence or absence of venom poisoning

Group A—Cases with no evidence of venom poisoning	32
Group B—Cases with definite evidence of venom poisoning	23
Total number of cases	55
Number of fatalities	7
Over-all mortality rate, 7 in 55	12.7%
Mortality rate in cases with venom poisoning, 7 in 23	30.4%

From a prognostic and therapeutic standpoint, as well as statistically, it is important to differentiate between those snake bites which will be essentially innocuous and those bites in which venom poisoning will be manifest. It is our conviction that a correct evaluation of the clinical course can be obtained in most instances by a minimum of laboratory work in conjunction with what information can be obtained with regard to the offending snake.

Group A, then, includes the fourteen patients in this series that were completely asymptomatic following the snake bite. Fourteen more were placed in this group because they had minimal swelling only. A few did have associated pain, but it was not considered to be due to toxic venom injection. Four cases

were questionably included in Group A. Two of these were admitted for the first time a few days after being bitten, with a secondary infection at the location of the bite. The other two developed secondary infection after hospital admission. It was impossible in these cases to show clearly that venom-induced tissue necrosis was the underlying element of the secondary infection.

Of particular importance are the analyses and conclusions to be drawn from the twenty-three cases in Group B. These cases have been classified as depicted in Chart 4. The clinical picture of category 1, swelling of the affected part with lymph node enlargement and pain, may well be challenged as evidence of venom poisoning. However, the local lymphatic involvement became manifest too quickly following the bite to be explained on an inflammatory basis due to secondary infection at the wound site with regional extension. The case of numbness was a known coral snake bite, but no other symptoms were present. A second known coral snake bite victim suffered no reaction whatever.

CHART 4

Classification of symptoms of venom poisoning (Group B)

1. Swelling of limb with lymph node enlargement and pain	3	4 cases
2. Numbness	1	
3. Proteolysis	12	19 cases
4. Bleeding	17	
5. Bleeding and hemolysis	12	

Categories 3, 4, and 5 are concerned with undeniable evidence of venom poisoning. Of the nineteen cases in these three categories, nine were bitten by *B. atrox*, and one, a case with bleeding from the gums only, was bitten by *B. schlegelii*. No snakes were available for identification in the remaining nine cases. It should be noted that seventeen of the nineteen cases in categories 3, 4, and 5 had bleeding, and that twelve of them also had hemolysis. Proteolysis was present in more than fifty per cent of these cases, but in most instances was not severe. One toe was amputated because of extensive local sloughing.

The mortality rate of seven in twenty-three, or 30.4 per cent, in Group B, is a good deal higher than that for the over-all mortality in our series and in other publications, since the fatal cases in this series, with but one exception, were not admitted early enough to be treated effectively.

SUMMARY OF AUTOPSY FINDINGS

Chart 5 summarizes the essential autopsy findings in the seven fatal cases. It will be noted that hemorrhage is a common factor in all cases. It varies only in extent and location. Cases 1, 3, and 6, are noteworthy because they exhibit severe hemorrhage within the central nervous system, which was the cause of death. Hemorrhage may occur early or late in the clinical course. Cases 4 and 7 did not have central nervous system bleeding. In all probability the extensive oozing of blood and serum, accompanied by hemolysis, along with other unknown factors, caused fatal shock. Case 2 had rather meager autopsy findings,

CHART 5

Summary of autopsy findings

CASE NUMBER	AGE SEX	SURVIVAL TIME (HOURS)	LOCAL REACTION	HEMORRHAGIC PHENOMENA	OTHER FINDINGS	CAUSE OF DEATH
1*	40 M	56	Local necrosis right ankle	Retroperitoneal hemorrhage Perinephritic hemorrhage Congestion of bladder and bowel Blood in spinal fluid Caudate nuclear hemorrhage Blood in lateral ventricles		Hemorrhage into Vital Center
2*	55 M	58	Left foot bleeding	Hemorrhage at wound site Moderate visceral congestion		Shock
3	50 M	5	Left foot hemorrhagic necrosis	Bleeding into oral cavity Blood in spinal fluid Cerebral softening	No blood clots. Known *Fer de lance*	Hemorrhage into Vital Center
4	25 M	100	Right foot extensive hemorrhagic necrosis to psoas muscle	Retroperitoneal hemorrhage Congestion of renal pelvis Pericardial hemorrhages Thoracic muscle hemorrhage Bowel hemorrhage Blood in spinal fluid		Extensive hemorrhage with shock
5	52 M	21	Right wrist hemorrhagic edema	Bowel hemorrhage Leg muscle hemorrhage Edema, congestion, enlargement of axillary lymph nodes	Known *Fer de lance* Guinea pig died after injection of suction fluid No clotting of blood	Shock?
6	25 M	54	Right foot hemorrhagic necrosis to thigh	Retroperitoneal hemorrhage Bowel hemorrhage Pulmonary hemorrhage Sub-pleural hemorrhage Sub-arachnoid hemorrhage Blood in spinal fluid Right occipital hemorrhage Cerebral softening Inguinal lymphadenopathy		Hemorrhage into Vital Center

7	27 F	21	Second toe left foot hemorrhagic edema to groin	Retroperitoneal hemorrhage Hemothorax Hemorrhagic pericardial effusion Perirenal hemorrhage Scalp hemorrhage Bowel hemorrhage		Extensive hemorrhage with shock

* Cases previously reported, Bates, L. B., Snakes and Snake Bite Accidents of the Panama Canal Zone, Bull. A.I.A., Vol. 2, p. 31, June 1928.

but the clinical history is one of bleeding from the wound area that was severe and prolonged, and it is assumed that he died of shock.

Case 5 is of interest. Autopsy examination revealed scattered hemorrhages in addition to hemorrhage and edema of the bitten part and enlargement of the regional lymph nodes, but there was no hemorrhage into a vital center. This victim of a *fer de lance* bite was a well-known herpetologist who had been bitten on some twenty previous occasions by many different species. The fatal bite was received while milking a starved snake. He was treated with tourniquet, suction, incisions, and antivenin. In view of the known sensitivity of his skin and respiratory passages to dried *fer de lance* venom and previous injections of antisera, the possibility of an anaphylactoid reaction must be considered, though it is likely that death was due to venom toxicity.

CASE PRESENTATION AND DISCUSSION

Case History, M. A. S. The patient, a 34-year-old colored female, was bitten on the left ankle by an unidentified snake about one p.m., 27 July 1951, while walking down a jungle trail. She applied a vine tourniquet, and was found later by a police patrol on a jungle road. At that time she was oozing blood and serum from the wound and from the mouth. She was admitted to Gorgas Hospital at four-fifteen p.m., some three hours after being bitten. Course in Hospital:

27 July 1951

4:15 p.m.

Examination—Bleeding from gums and buccal mucosa, BP 130/70.

Laboratory—Prothrombin less than 1% of normal. Urine bloody. Coagulation time, 14 minutes. Bleeding time, 1 minute. RBC 4,240,000, Hb. 11.0 gms., WBC 20,000 90% N.

Treatment—2 vials polyvalent Bothropic antivenin intra-muscular and 2 vials injected at wound site. Penicillin, Vitamin K, 500 cc. fresh whole blood started incisions with suction and culture of wound.

6:50 p.m.

Examination—Oozing profusely from mouth, abdomen rigid, urine bloody, hematoma developing on left anterior chest wall, bleeding from needle punctures and wound. Left leg discolored and edematous.

Laboratory—Prothrombin less than 1% of normal. Coagulation time, infinity. Bleeding time, 4 minutes. Urine bloody.

Treatment—700 cc. fresh whole blood. Decision made to freeze the leg and to place arterial constricting tourniquet.

28 July 1951

7:30 a.m.

Examination—Oozing from mouth, wounds, all needle punctures, urine bloody, still signs of peritoneal irritation.

Laboratory—Prothrombin, less than 1% of normal. Coagulation time, 52 minutes.

Treatment—2 ampules of antivenin (10 a.m.), 1000 cc. whole blood.

2:45 p.m.

Laboratory—RBC, 3,700,000, Hb. 10.8 gms.

Treatment—1000 cc. fresh whole blood.

6:00 p.m.

Laboratory—RBC, 2,470,000. Urine bloody.

Treatment—500 cc. fresh whole blood, 2 ampules antivenin.

10:30 p.m.
Laboratory—RBC 2,860,000, Hb. 10 gms.
Treatment—400 cc. fresh whole blood.
29 July 1951
6:00 a.m.
Examination—Abdomen softer, slight oozing from wound, chest hematoma no larger, urine bloody.
Laboratory—RBC 2,600,000, Hb. 10 grams.
Treatment—2 ampules antivenin, 500 cc. fresh whole blood.
11:00 a.m.
Laboratory—Prothrombin, less than 1% of normal. Coagulation time, beginning to show clot tendency.
Treatment—1000 cc. fresh whole blood given during afternoon.
7:30 p.m.
Laboratory—RBC 3,350,000, Hb. 10.5 gms. Coagulation time, 4.5 minutes. Bleeding time, 2.5 minutes. WBC 20,000.
30 July 1951
Examination—Oozing has stopped, feeling better.
Laboratory—No further drop in blood count, and coagulation time normal.
Treatment—500 cc. fresh whole blood.
31 July 1951—Leg amputated above knee, uneventful recovery.

The above case is cited as an example of the rapid and severe effects that can be seen in ophidism. In a very short time the patient noted hemorrhagic tendencies and these were moderate in degree on admittance to the Hospital three hours after the bite. Within another three hours the hemorrhagic tendencies were marked, and the clinical condition of the patient was critical. Coupled with the knowledge that venom in toxic dosage has been retrieved as long as fourteen hours after introduction (Jackson, 1929) was the strong possibility of hemorrhage into the central nervous system. It was deemed essential that a method of eliminating further venom absorption be employed. Freezing the limb and a constricting tourniquet was the method chosen. The authors are not advocating amputation of limbs affected by snake bite, but do consider that such a procedure may be necessary in some rare instances (Allen, 1939).

The hemolytic factor became active in this case some twenty hours after the injection of venom. The hemolysis was severe, rapid and most marked thirty hours after the bite. Forty-eight hours after the bite the coagulation property of the blood began to return, and approximately six hours later the hemolytic activity was no longer demonstrable and the coagulation had returned to normal. Seventy-two hours after the bite the patient definitely looked and felt as if the period of venom effect had passed. The hemolysis was successfully combatted with fresh whole blood. The factor of shock or of renal shut-down was never manifest. The failure to observe a patient and adequately transfuse in this period could well result in a fatal outcome.

Proteolysis was present in the amputated specimen. However, the severity and extent of proteolysis is difficult to evaluate in a limb that has been subjected to occlusive tourniquet and refrigeration. A few cases of extensive sloughing due to the necrotizing influence of the venom with or without secondary infection have been seen (Clark, 1952; Picado, 1931). However, proteolysis is of

secondary importance in the light of the more serious effects of anticoagulation and hemolysis.

The above case appears to be unusual. It is the most severe nonfatal example of venom poisoning encountered at Gorgas Hospital. Others have had the elements of anticoagulation, hemolysis and proteolysis present, but in lesser degree, and ordinarily only one of these features is outstanding.

GENERAL DISCUSSION

Snake bites on the Panama Canal Zone seem to fit into two main groups, those demonstrating no effects of venom injection, and those with a clinical picture recognizable as venom poisoning. The problem of snake bites in the Panama area is concerned primarily with *B. atrox* and to a much lesser degree with the smaller members of the genus *Bothrops*. Bites by other genera of poisonous snakes are uncommon.

The exact composition of snake venom is not known, though a great deal has been learned. Ganguli (1946) lists six main toxic principles that occur in the various types of venom. Some are still somewhat theoretical and may well represent multiple effects of a single substance. It has been suggested that some of the toxic chemical substances of the venom complex are held in common by many species. However, it has been shown that in the case of certain European vipers the paraspecific action of Bothropic and Crotalic antivenin is inferior to the specific antivenins (Schöttler, 1951). One must conclude that the similarities in the toxic manifestations produced by many snake venoms does not mean that the venoms are chemically or antigenically identical. With regard to the Bothropic venoms there are at least three main toxic effects produced. These are tissue necrosis, anticoagulation of the blood, and hemolysis of the red blood cells. In the cases studied we did not observe any evidence of a neurotoxic principle.

The rather rapid onset of anticoagulant effects on the blood following injection of venom is of no minor importance. In several cases the prothrombin approached zero per cent of normal within three hours after the bite. The exact mechanism by which venom destroys prothrombin is not known. Vitamin K does not appear to have any effect upon prothrombin once the venom has started to exert its detrimental influence. Best and Taylor (1943) state that there is a chemical change in the thromboplastin following venom injection. Closely associated with the disorder of the prothrombin element of the clotting mechanism is the marked increase in coagulation time. The full effect of loss of coagulability is not manifest until a few hours after the prothrombin approaches zero per cent of normal. It is not likely that a "lag phenomenon" is present but rather, as other elements of the coagulation mechanism in addition to prothrombin are destroyed, there is complete loss of blood coagulation. It can be said, however, that the laboratory determination of coagulation time and of prothrombin time is a necessity in snake bite cases where there is a question as to the hematologic effects of the venom. These are procedures which do determine early blood changes, and are warning signs of severity if markedly deviated from normal. It would appear that if after twenty-four hours these tests still show no tendency

to revert toward normal, increased amounts of antivenin should be given (Engler, 1951).

Kauer, Bird and Reznikoff (1943) showed in their experiments with dogs injected with *fer de lance* venom that there was a marked lengthening of the clotting time, and a marked decrease of the prothrombin level. They also noted that the fibrinogen level was lowered. Rabbit antivenin which they produced not only protected the dogs against lethal doses of the venom but also modified the venom effects on clotting time and the fibrinogen and prothrombin levels. Castro (1942) stresses that both fibrinogen and globulin are destroyed by Bothropic venom, the prothrombin time changes being an indication of the severity of this destruction. Fresh whole blood transfusions are valuable measures which aid in fibrinogen and globulin replacement.

In view of Duran-Reynals' work (1939), it is highly probable that closely associated with the anticoagulation effects of venom toxicity is an increase in capillary permeability. Though this factor has not been fully proven, its presence in addition to the loss of blood coagulability would adequately explain the marked oozing of serum and blood cells seen in snake bite victims and most often described as bleeding or hemorrhage.

In many instances, before the anticoagulation effect has begun to regress to any appreciable degree, hemolysis appears. The exact mechanism of red blood cell destruction is not known. It has been shown that some venoms contain a principle that is able to remove unsaturated fatty acids from the lecithin component of red cells and plasma alike (Best *et al.*, 1943). The resultant product is hemolytic. Without doubt other factors, not well understood, play important roles in causing hemolysis. Schöttler (1951) feels that there may be several different hemorrhagins present in snake venoms that act upon different organs.

The degree of hemolysis varies markedly and appears more apt to be of a lesser degree than the earlier effect of anticoagulation. This variation may be on the basis of venom difference, or perhaps variation of human reaction to venom. However, since our observations have been largely limited to patients who have received antivenin, it is well to consider the possibility that antivenin, even in relatively small amounts, prevents a full-force display of venom toxicity. Hemolysis often is rapid in onset, severe and may last for more than twenty-four hours. We do not hesitate to state that this can be combatted adequately with fresh whole blood transfusions. So far, in the cases of venom poisoning that have survived, we have had no shock or renal damage. The inherent ability of the hemorrhagic disturbances of many snake venoms to cause death cannot be ignored or considered lightly.

The shock picture of venom poisoning has been emphasized in recent publications on snake venom and its properties (Schöttler, 1951; Rocha *et al.*, 1949). That tissue damage and destruction with its consequent liberation of breakdown substances, including histamine, have their effect, cannot be denied. Rocha e Silva, Beraldo, and Rosenfeld (1949) have recently described a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms, which has been called bradykinin. It is plausible to assume that

anticoagulation and capillary permeability with their consequent oozing of serum and blood cells closely followed by hemolysis, together with other as yet relatively obscure factors, form the etiologic background of shock.

Proteolysis varies widely. There may be no tissue necrosis at all, or it may be the most marked feature of venom toxicity. Snake venom is thought to produce tissue destruction by its enzymic activity, and venom seems to spread through tissue spaces readily (Duran-Reynals, 1939). Allen (1939) observed that when the proteolytic action of venom is marked, the hemorrhagic and hemolytic aspects seem to be of a lesser degree. Minimal to moderate proteolysis was seen in over fifty per cent of our cases of venom poisoning, and in nearly all cases the hemorrhagic or hemolytic effects were more marked than the tissue destruction at the location of the bite. However, there is evidence of the local tissue binding of venom (Allen, 1939). One of our cases, in which there was a moderate anticoagulation disorder, required local excision of a necrotic area. He returned five weeks after the bite with necrosis in the tissue immediately adjacent to the line of excision, and a sinus tract between muscle tendons had developed. Picado (1931) reported experimental evidence of the freeing of toxic venom days after the injection with local necrosis and hemorrhage in the case of Bothropic venom. In the ordinary case treated with adequate amounts of antivenin, this reaction should not be expected.

There remains a great deal to be learned with regard to the mode of effects of ophidic venom. The wide variation of species and venoms in different localities complicates the problem. Many of the most important aspects of venom poisoning have not been thoroughly studied. Why prothrombin is decreased so markedly is not known. This effect seems much more rapid and less prolonged with snake venom than with dicoumarol. The literature contains little information concerning the decrease in fibrinogen level as a result of venom poisoning. The exact underlying mechanism causing complete loss of blood coagulation, as tested by the usual method of Lee and White, has not been satisfactorily explained. We do not know to what degree the severity or the duration of the anticoagulation disorder is affected by fresh whole blood transfusions. Any direct relationship between the loss of normal blood coagulability and hemolysis is not apparent. The factors in addition to decreased coagulation, capillary permeability and hemolysis that combine to produce shock have not been thoroughly explored.

The exact mode of action of antivenin is not understood. Schöttler (1951a) in his experimentation concluded that none of the antivenins studied had any selective neutralizing property against the hemorrhagic principle of Bothropic venoms. He further states that the toxic factors of the venom complex play no integral role in immunization and may not be antigenic. In a subsequent report (1951), however, he adds that in one way or another the toxicity must be linked to the antigen as it is neutralized when venom and antivenin (antigen and antibody) combine. The apparent lack of a standard viewpoint on the mechanism of antivenin production and its mode of action may mean that the toxic principles in venom are so complex and variable that no conclusions of specificity can be made on the basis of information now available.

The value of antivenin, however, cannot be assailed. Lives are saved by its use. Experimentally, life was saved by the use of antiserum after lethal doses of *fer de lance* venom had been given dogs (Kauer *et al.*, 1943), and Picado (1931) obtained the same results with rabbits. Schöttler (1951a) points out that in Brazil the titer of the antivenins available is sufficient to cure the average snake bite, except that of the tropical rattlesnake, for which species the antivenins presently available have not reached a stage of curative efficacy. In Costa Rica, however, the once deadly tropical rattlesnake bite is being treated with antivenin alone, with no recent fatalities (Trejos-Willis, 1952). The Chiriqui Land Company Hospital at Almirante, Panama, treats on the average twenty-five Bothropic snake bites a year, and there have been no fatalities in a period of four years (Engler, 1951).

It is common knowledge that statistics can often be misleading. The mortality rate of 30.4 per cent in cases demonstrating venom poisoning may at first glance appear somewhat exaggerated. However, Basu (1939) reported briefly on twenty cases of viperine bites in Calcutta, India, in which the mortality was sixty per cent. It must be concluded, therefore, that a snake bite victim is a potential fatality and should be regarded as such. However, with proper care and therapy the mortality rate can be made to approximate zero.

TREATMENT

The treatment of snake bites does not pose an insurmountable problem. Great strides have been made since the days when no clear-cut procedure was advocated. The importance of education of the general population and especially those who find themselves potentially exposed to poisonous snakes cannot be overemphasized.

The application of the tourniquet should be advocated in all cases of bites on the extremities. It should be applied in such a manner as to obstruct the venous and lymphatic flow but not the arterial circulation. Suction is probably more important. A small piece of thin rubber sheeting, to be used between the mouth and the wound, is easily carried and highly recommended. Suction, if it is to be used to the greatest advantage, must be started immediately following the bite and must be vigorous.

Some hold that incisions allow an increased tissue surface to absorb the venom, and since the anatomy of the fangs is such that the actual venom deposition site is not necessarily below the surface puncture wounds, incisions at that location are of dubious benefit (Clark, 1942) Others feel that both incision and suction should be employed, to extract as large a quantity of the injected venom as possible (Jackson, 1944). Jackson's report (1929) that toxic venom though diluted with tissue serum, has been removed as long as fourteen hours after a rattle-snake bite, shows that at least in some instances suction and incision combined remove the undesired toxic element. It would seem that the best procedure is suction immediately following the bite and, after medical aid has been obtained, continued suction along with incisions over the fang marks. Additional incisions subcutaneously around the edge of the advanced swelling, may be used to advantage (Ganguli *et al.*, 1946; Jackson, 1944).

The use of antivenin in addition to tourniquet, suction and incisions is a necessity. It may well be the life-saving element of therapy, particularly when venom is but partially removed. Several types of antivenin are available. Depending upon the circumstances and the type of venomous snakes in the area one may choose the antivenin best suited for his needs. In the United States, Crotalic antivenin for rattlesnakes and also antivenin for moccasin and copperhead snakes is available and efficacious. In regions where the Cobra group is important specific antivenin can be obtained. In the tropics of Central and South America the most important bites are by serpents of the genera *Bothrops* and and *Crotalus*. In general *Micrurus* rarely bites man and *Lachesis muta* is scarce and few bites are recorded, at least in Panama. Antivenin is available for the two last mentioned species.

In Panama a polyvalent Bothropic antivenin will suffice in most instances. In other regions, Costa Rica for example, it is best to use a combination of polyvalent Bothropic and the Crotalic antivenin, since the incidence of bites there by the tropical rattlesnake is significant. The antivenin in use at Gorgas Hospital is a product of Vital Brazil-Niteroi or of the Instituto Butantan in Brazil, and 10 cc. is described as neutralizing approximately 15 mg. of Bothropic venom. This means that high amounts of antiserum must be given in the majority of cases since many of these reptiles are capable of injecting as much as 160 mg. of venom. Antivenin comes in easily carried vials and should be part of the equipment carried on jungle trips.

In view of the unknown amount of venom that is injected it would seem advisable in all cases of suspected venom poisoning that as a minimum one ampule of antivenin should be injected every hour over a four hour period. This will allow for a sufficient time period of observation and study and the general severity of the situation can be ascertained. If symptoms persist at this time at least one ampule should be given every four hours for one or two days. If only edema remains it is well to continue treatment for twenty-four to forty-eight hours, giving one ampule every twelve hours. If it is thought that the venom dosage is sizable it is well to inject several ampules at the onset of treatment. In general smaller individuals require an increased quantity of antivenin.

Antivenin must be given in copious amounts and only in rare instances is it necessary to give it by other than the intramuscular route. All patients should have a routine skin sensitivity test.

SUMMARY

Nearly all poisonous snake bites in the Canal Zone and Panama are by the genus *Bothrops*, and the majority are by *B. atrox*. The incidence is low and the mortality in 23 cases in which there was evidence of venom poisoning was 30.4 per cent.

Bothropic venom may produce hemorrhagic tendencies, hemolysis and proteolysis, although all these effects may not be manifest in a given case. Anticoagulation and bleeding, with hemolysis, usually began in the series reported within 20–24 hours after the bite, and began to clear in about 48 hours. The degree to

which antivenin and transfusions affected this sequence is not known. Proteolysis was not an important factor at this stage.

Seven fatal cases are reported, with shock and hemorrhage into the central nervous system as the principal causes of death. One severe case which recovered is presented in detail. Suggestions are given as to the best form of treatment, based on these cases and those reported by other authors. Certainly the unknown aspects of venom poisoning, the factors affecting shock production and the evaluation of therapeutic measures need more investigation and accurate reporting by those in a position to do so.

REFERENCES

1. Allen, F. M. 1939, Observations on local measures in the treatment of snake bite, *Am. J. Trop. Med.* **19:** 393–404.
2. Basu, U. P. 1939, Observations on scorpion-sting and snake-bite, *Am. J. Trop. Med.* **19:** 385–391.
3. Best, C. H., and Taylor, N. B. 1943, The Physiologic Basis of Medical Practice, Williams and Wilkins Co., Baltimore, p. 84 and p. 152.
4. Clark, H. C. 1942, Venomous snakes. Some Central American records. Incidence of snake bite accidents, *Am. J. Trop. Med.* **22:** 37–49.
5. Clark, H. C. 1952, Personal communication.
6. Duran-Reynals, F. 1939, A spreading factor in certain snake venoms and its relation to their mode of action, *J. Exp. Med.* **69:** 69–81.
7. Engler, Gustave 1951, Personal communication.
8. Ganguli, S. K., and Napier, L. E. 1946, Snakes and snake-bites, in Principles and Practice of Tropical Medicine, The Macmillan Co., New York, p. 836–859.
9. Castro, Guillermo 1942, Accion del veneno en la sangre de la victima de Bothrops, *Rev. Med. Costa Rica* **5:** 13–16.
10. Jackson, Dudley 1929, Treatment of snake bite, *So. Med. J.* **22:** 605–607.
11. Jackson, Dudley 1944, Management of snake bite, in Clinical Tropical Medicine, edited by Berkovitz, Z. T., *et al.*, Paul B. Hoeber Co., New York, p. 881–884.
12. Johnson, Carl M. 1952, Personal communication.
13. Kauer, G. L., Bird, R. M., and Reznikoff, Paul 1943, The clotting action of fer-de-lance venom, *Am. J. Med. Sc.* **205:** 16–24.
14. Picado, C. 1931, Serpientes Venenosas de Costa Rica, Imprenta Asina, pp. 101, 119, 205.
15. Schöttler, W. H. A. 1951, Toxicity of principle snake venoms of Brazil, *Am. J. Trop. Med.* **31:** 489–499.
16. Schöttler, W. H. A. 1951a, Antigen-antibody relations in the present antivenin production of Brazil, *Am. J. Trop. Med.* **31:** 500–509.
17. Schöttler, W. H. A. 1951, Para-specific action of Bothropic and Crotalic antivenins, *Am. J. Trop. Med.* **31:** 836–841.
18. Rocha e Silva, M., Beraldo, W. T., Rosenfeld, G. 1949, Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and by trypsin, *Am. J. Physio.* **156:** 261–273.
19. Trejos-Willis, Alfonso 1952, Personal communication.

THE L-C TREATMENT OF VENOMOUS BITES OR STINGS

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The so-called "cut-and-suction" treatment when used on venomous stings is not very effective. This fact we learned at an early date in our experiences with poisoning caused by the two lethal scorpions of the Southwest, i.e., *Centruroides sculpturatus* Ewing and *C. gertschi* Stahnke. During a twenty year period in Arizona, these two animals were responsible for more than twice as many deaths as all other venomous animals put together, including rattlesnakes, black widow spiders, Gila monsters, and venomous insects (Stahnke, 1949). In our first observations on the effect of rattlesnake venom, we were shocked at the suffering that patients had to undergo even though the best known treatments were administered. Out of these experiences grew the treatment now known as the L-C method, i.e., ligature and cryotherapy.

In treating a patient for venom poisoning two forces must be taken into consideration, the chemical effect of the toxin on the animal tissue and the bacterial action of bacteria possibly introduced by the bite or sting. If both of these are introduced in small enough quantities, the natural defensive forces of the body can neutralize their effects. The L-C method takes into account the above principle.

THE L-C TREATMENT

Let us assume that a person is stung or bitten by a venomous animal on one of the extremities, e.g., the tip of the index finger. Serious consequences may be averted by doing the following:

1. Place a ligature (a tight tourniquet) *at once* between the site of the bite or sting and the body, but *as near the point of entrance* of the venom as possible. A heavy piece of string or shoe-lace will serve very well as a ligature. A handkerchief, bandage, or necktie is not very suitable but will be better than nothing. The ligature can be effected by wrapping the string three or four times around the finger and then twisting it tightly, to the point of pain, by means of a small stick, nail, or other suitable object. *Do not make any incisions at the site of the bite or sting.* This is unnecessary and is only inviting more difficulties.

2. Now place a piece of ice on the site while preparing a suitable vessel of crushed ice and water.

3. Place the entire hand in the iced water prepared. In general, immerse the member bitten or stung in the iced water *well beyond the location of the ligature.*

4. After the hand, or other member, has been in the iced water for five minutes the ligature must be removed, but the hand is kept in the iced water for at least two hours. If the attacking animal was one of the two lethal scorpions, no serious effects will be experienced after this time. For the Gila monster, six to seven hours was found necessary. For rattlesnakes or other pit-vipers, the time element will depend upon the size of the attacking animal. At least six hours will be necessary in order to prevent serious destruction of tissue. For a large

rattlesnake the patient may have to be treated for 14 to 20 hours. During this time it is imperative that the patient be kept warm so that circulation through all parts of the body except the poisoned member be good. If the individual is bitten on an area other than the extremities, a large ice-pack of finely divided ice may be used. If ethyl chloride is available, the temperature of the involved tissue can be reduced more quickly. An area of approximately three inches around and including the site of the bite or sting can be sprayed, taking care not to freeze the tissue. This can be accomplished by spraying only to the point of frost formation. After this has disappeared, the process should be repeated. When the area has become thoroughly chilled, it can be held in that condition with an ice-pack. In case of snake poisoning, where the swelling has advanced beyond the site of the bite, the appendage should be immersed in the iced water, or packed in crushed ice, well above the area of swelling.

In using cryotherapy, the initial pain experienced during the first five mintes must not be confused with the action of the venom. Likewise, there is no danger of frost bite where iced water with nothing added is used. When going into wild country, the camper, hunter, or scientist can carry several metal tubes of ethyl chloride to be used in lieu of ice. If the procedure outlined above is used, one tube can be made to last about thirty minutes. Thus two tubes, under normal conditions, would give sufficient time to get to ice and/or a physician (Stahnke, 1949a).

BENEFITS OF THE L-C METHOD

Venom action is chemical action. For every 10°C. rise in temperature, chemical activity is doubled; the converse is likewise true. A hand kept in iced water will have its temperature dropped from the normal body temperature of 37°C. to about 4–7°C. Consequently, the chemical activity at the site of injection is practically nil.

The chemical action of venom in the body becomes harmful only when the natural defenses of the body are overwhelmed. The ligature holds the venom at the site of injection until the temperature of that region can be dropped. This lowered temperature causes the tissues to contract and the capillaries and arterioles to constrict. Thus the venom is so held that it will enter the body very slowly and will be distributed throughout the body in an extremely dilute condition—too dilute to cause untoward effects.

Harmful bacteria are introduced with the bite of rattlesnakes and other biting venomous animals. In the presence of the venom and the normal body temperature these organisms thrive. When the body temperature is reduced by approximately 30°C., these organisms cannot multiply.

In addition to the above, we also have the benefit of the anesthetic action of the cold and the providing of ample time to administer antitoxin where such is available. However, death can be prevented with the L-C treatment alone if sufficient time is given for its use.

REFERENCES

STAHNKE, H. L. 1949, Scorpions, Arizona State College at Tempe, p. 7.
STAHNKE, H. L. 1949a, *Op. cit.*, pp. 19–21.

BOOKS RECEIVED

Note: Books received for editorial consideration will be intermittently listed. This acknowledgment must be regarded as an adequate expression of appreciation for the courtesy of the author or publisher. Selections will be made for review in the interest of our readers.

Monographs in Medicine. Series 1. Ed. by William B. Bean, M.D. (State University of Iowa). Associate Editors: Morton Hamburger, M.D. (University of Cincinnati), John A. Leutscher, M.D. Stanford University) and Steward Wolf, M.D. (University of Oklahoma). 655 pp., illustrated. Baltimore, The Williams & Wilkins Co., 1952. Price $12.00.

Nomenclátor de las Enfermedades Transmissibles y Símili-Transmissibles. By Luis E. Nájera, Profesor de Epidemiologia de la Facultad de Higiene y Medicine Preventiva, Universidad Nacional de Tucumán, Rep. Argentina. 257 pp. Santa Fé, Argentina. Imprenta de la Universidad Nacional del Litoral. 1952.

Methods of Trachoma Control. Expert Committee on Trachoma. First Report. W. H. O. Technical Report Series No. 59. (Available also in French). 22 pp. World Health Organization, Geneva, or Columbia University Press, International Documents Service, 2960 Broadway, New York 27, N. Y. 1952. Price 1/3; $0.15; Sw. fr. 0.60.

Ground Equipment and Insecticides for Mosquito Control, Bulletin No. 2. American Mosquito Control Association, Inc. By E. F. Knipling. 116 pp. Yapank, N. Y., C. T. Williamson, Business Manager. 1952. Price $2.00

Malaria. Basic Priciples Briefly Stated. By Paul F. Russell, M.D., M.P.H., Division of Medicine and Public Health of The Rockefeller Foundation; Consultant to Surgeon General, U. S. Army; formerly Chairman Expert Committee on Malaria of W.H.O. 210 pp. 64 illustrations, 3 in color. Published simultaneously by Blackwell Scientific Publications, Oxford, England; Chas. C. Thomas, Springfield, Illinois, U.S.A. and Ryerson Press, Queen St. West, Toronto 2, Canada. Price $7.75; 35/–

Impletoltherapie und andere Neuraltherapeutische Verfahren. Grundlagen und Technik. By Dr. med. Walter Huneke. 126 pp. Stuttgart, Hippokrates-Verlag Marquardt & Cie. 1952. Price DM 12.50; English 9.50.

Joint Expert Committee on the Physically Handicapped Child, First Report. W.H.O. Technical Report Series, No. 58. 26 pp. Geneva, W.H.O., Palais des Nations, 1952. Price 1/6; $0.20; Fr. fr. 65; Sw. fr. 80.

BOOK REVIEWS

Médecine Tropicale, dans la Collection Médico-chirurgicale a révision annuelle (Directeur général: Pasteur Vallery-Radot), par Marcel Vaucel, Inspecteur Général des Instituts Pasteur d'Outre-Mer, ancien directeur du Service de Santé au Ministère de la France d'outre-Mer., avec 14 collaborateurs. Deux volumes à reliure mobile, 1968 pp., 243 figures, 5 planches hors-texte en couleurs. Paris—VI^e, France. Éditions Médicales Flammarion, 22, rue de Vaugirard. Prix (2 vols.), Fr. francs 12,000. 1952.

This text on Tropical Medicine is of special interest for the following reasons: it is new; it is a collaborative enterprise on the part of an experienced leader in tropical medicine and fourteen specialists in particular areas of the field, and it is loose-leaf-bound, providing opportunity for annual revision.

Volume I is divided into three parts, viz., helminthic infections (pp. 9–352), protozoan infections (pp. 355–828) and spirochetoses (pp. 831–947). Volume II includes virus infections (pp. 959–1138), rickettsioses (pp. 1141–1226), bartonellosis (pp. 1229–1242), bacterial diseases (pp. 1245–1547), mycoses (pp. 1551–1592), avitaminoses (pp. 1595–1725), diseases

due to arthropods (pp. 1729–1736), myiasis (pp. 1739–1748), diseases of diverse etiology (pp. 1751–1814), poisoning caused by animals (pp. 1817–1850), and by plants (pp. 1853–1889), effects of tropical climate (pp. 1893–1906), and a subject index (pp. 1909–1932). Each chapter is provided with a bibliography. A Table of Contents for Volume I is placed at the end of the volume and a combined Table of Contents for both volumes at the end of Volume II.

The following questions are relevant to an evaluation of the book. Is the information comprehensive, up-to-date, accurate, well-balanced and coördinated? Are the recommended therapeutic and prophylactic procedures efficacious and practical? Are the reference citations adequate? Is the text well illustrated? Is it relatively free of typographical errors? Is the format pleasing and is the printed page easy to read? And are the volumes substantially bound?

The section on helminths consists of notes on minor tapeworm infections (hymenolepiasis and sparganosis, with no mention of taeniasis, cysticercosis or hydatid disease), extensive coverage of trematode infections and comprehensive presentation of ancylostomiasis, ascariasis, strongyloidiasis and the filariases (but no consideration of whipworm infection). The pages devoted to bilharziasis contain much valuable material but it is difficult to sort this out from the numerous inaccuracies, especially on geographical distribution. The distribution map (Fig. 1, p. 26) is misleading, since it assigns this disease to many areas where indigenous infection does not exist and fails to include several important areas of endemicity. The misspelling of names of many investigators cited in the text and in the bibliography leads one to wonder how familiar the author is with the literature on bilharziasis. Too much space is allotted to hepatic and intestinal distomiasis and possibly not enough to paragonimiasis. In these chapters there are numerous orthographic errors, especially of etiologic agents.

The nematode infections are on the whole very well presented. But one looks in vain, among the many chemotherapeutics listed, for the author's recommendations of the most satisfactory anthelmintic medication. The chapter on Bancroft's filariasis is too generously illustrated with photographs of the advanced chronic elephantoid stage, but provides little basic information and no illustrative material on the development of this disease and its early acute stage.

Amebiasis is presented in considerable detail. The subject is skillfully handled and the illustrations are good. All of the important antiamebic drugs, including antibiotics, are listed and from these the reader is allowed to make his own selection. The bibliography on this infection is extensive but contains very few references to critical evaluation of the more effective amebicides.

The chapters on African trypanosomiasis and malaria are among the most valuable and comprehensive contributions in Volume I. They are authoritative and are well documented. Cutaneous leishmaniasis receives adequate treatment but kala-azar probably deserves more detailed consideration. The chapters on spirochetal diseases (relapsing fever, leptospirosis, yaws, pinto, etc.) are very effectively written. They contain up-to-date practical information.

The first chapter in Volume II is a well-balanced monograph on yellow fever, covering historical landmarks, the causative virus, 17D and Dakar types of vaccine, clinical aspects of the disease, and extensive data on prophylactic measures (anti-mosquito campaigns and vaccination). Dengue, sand-fly fever and other tropical fevers of viral origin transmitted by arthropods are more briefly presented. Rabies, anterior poliomyelitis, the encephalitides, ornithosis, infectious hepatitis and trachoma are likewise included as important viral diseases prevalent in the Tropics.

The rickettsioses are first outlined by groups, then considered individually in more detail, viz., scrub typhus, tick-bite fever of Africa, Kenya typhus, Congo "red fever," African savanna typhus, Rocky Mt. spotted fever, rickettsialpox, Q fever, Queensland tick typhus, and, probably all too summarily, classical and murine typhus. No mention is made of enzoötic foci of scrub typhus in the Philippines. The trombiculid vectors of this disease

are likewise not critically evaluated (table, page 1149). The chapter on bartonellosis lists not only Peru, Colombia and Ecuador, but also Chile, Bolivia and Guatemala as countries having endemic foci.

Among the bacterial diseases there is an excellent monograph on cholera. Today, according to the author, epidemics of this disease can be rapidly controlled except in certain areas like the river deltas of India, where climatic, physiologic and social factors allow it to persist. Shigellosis is also well presented. Considerable space is justifiably given to plague. This chapter bears intrinsic evidence of having been written by an expert and is both informative and stimulating. The only modern raticide not mentioned is Warfarin. In the chapter on leprosy treatment is considered to be as much a sociologic and group-hygiene problem as one of chemotherapy. Chapters on tularemia, meloidosis, desert sore, granuloma inguinale, as well as lesions due to staphylococci, pneumococci, Brucella and meningococci provide useful supplementary information for the physician in the Tropics. Although typhoid and paratyphoid fevers constitute serious clinical and public health problems in warm climates, very scant consideration is given to the salmonellosis group of diseases.

The section on mycotic diseases first takes up the epidermophytoses, followed by the actinomycoses, maduromycoses, and the systemic diseases of fungous origin. Very few fungicidal remedies are provided, in refreshing contrast to the plethora of prescriptions in most texts on dermatology.

Nutritional deficiency diseases constitute a valuable section of the text. Almost a hundred pages are devoted to beriberi, which is regarded as the tropical deficiency disease of prime importance. Eradication of beriberi constitutes the "order of the day." This chapter is generously documented and contains an extensive bibliography. Tropical sprue, pellagra and pellagroid diseases are presented much less extensively.

The section on "Diseases due to Arthropods" is confined to infestation with the chigoe (*Tunga penetrans*) and porocephalosis, while myiasis is placed in a separate section "Diseases due to Flies." (Are flies no longer Arthropods?)

The section on "Divers Diseases" includes chapters on tropical phagedenic ulcer, "onyalai," ainhum, toxoplasmosis, tropical eosinophilia, tropical thrombophlebitis, sicklemia and sickle trait, and cancer in the Tropics,—truly an odd assortment indicating that entirely too little is known about the etiology and epidemiology of some of these clinical entities.

The two chapters on poisonous animals and animal venoms emphasize the importance of snake venenation. It is noted that only one of the two gila monsters (*Heloderma horridum*) is mentioned. Exotic plant poisons and poisoning are comprehensively considered. The final chapter, on the deleterious effects of tropical climate, is restricted to information relating to Equatorial Africa, the Sudan, Senegal and the Sahara. The brief development of this topic contains well-known but practical information and advice.

The planning, writing and synthesis of this new treatise on tropical medicine have been a tremendous task. On the whole, the result has been satisfactory and certain subjects merit particular praise. In some chapters there are factual errors, and there are hundreds of typographical misspellings, mostly, although not exclusively, in names of authors cited. Likewise the space allotted to some subjects is excessive, to other subjects it is too scant. Careful revision and meticulous proof-reading will eliminate most of these discrepancies in subsequent editions. The reviewer recommends Médecine Tropicale as a comprehensive, useful compendium, valuable not only to workers in French overseas territories but to students of medicine in other tropical areas of the world.

The text is clearly printed on good quality paper, and the illustrations are for the most part good, although not always well balanced with respect to textual presentation. The pages are sturdily bound in heavy linen-covered loose-leaf binders, designed for hard use in tropical climates.

ERNEST CARROLL FAUST

International Sanitary Regulations. Proceedings of the Special Committee and of the Fourth World Health Assembly on WHO Regulations No. 2. Official Records of the

World Health Organization No. 37. (Also published in French). 443 pp., Geneva, Switzerland, World Health Organization. Distributed by Columbia University Press, International Documents Service, New York City, 1952. Price $2.25, 16/3, Fr. fr. 720, Sw. fr. 9.–.

This volume contains the revision of the International Sanitary Conventions and their consolidation into a single set of regulations, applicable throughout the world to all forms of international transport, for the control of the six quarantinable diseases: plague, cholera, yellow fever, smallpox, typhus and relapsing fever. The last disease seems to have been added because of recent large epidemics, and of the danger of its spread through the Mecca Pilgrimage. It contains a record also of the successive steps leading to an accomplishment which must be considered one of the most important tasks yet undertaken by WHO. Valuable information is given on the considerations and principles on which the Regulations are based, summed up in "maximum security with a minimum of interference with international traffic."

Part I comprises the debates of the Special Committee and its Sub-Committee on the Mecca Pilgrimage, and emphasizes the opinion of the experts that "a community is more effectively protected against pestilential disease by its own public health service than by sheltering behind a barrier of quarantine measures." Part II contains the text of the regulations, with explanatory notes and an analytical index. For countries which have not explicitly "contracted themselves out" of the convention, the Regulations entered into force on October 1, 1952.

L. W. Hackett

Illustrated Map of the Anopheline Imagines of Indonesia (English Translation). Ministry of Health of Indonesia 2d edition; corrected and supplemented. 69 pp., Djakarta, 1949.

This guide to the identification of the adults of the anopheline mosquitoes of Indonesia, which apparently was prepared by W. J. Stoker and R. Waktoedi Koesoemawinangoen, is not set up as a conventional dichotomous key but is a list of species, each of which is accompanied by notes on the principal taxonomic characters and by diagrammatic illustrations of the palpi, proboscis, wing, hind leg and occasionally of other pertinent recognition characters. Notes on breeding habits and relation to malaria are included. The arrangement of the species is not entirely in phylogenetic order, but the species and subspecies are grouped in accordance with their similarity in appearance so that a person using the guide in identifying material can make comparisons easily. Seventy-six species and subspecies are considered. This reviewer does not agree with all the authors' interpretations regarding specific and subspecific status, and the fact that there is still basis for disagreement among taxonomists shows that much interesting research remains to be done in these anophelines with their insular distributions, especially if approached from the point of view of the "new systematics." The guide not only will be useful in identifying the anophelines of this region but through its comparative illustrations it points up the existence of many problems on the nature of mosquito species.

It seems a little unfortunate that this "map" was printed on paper measuring 11¼ by 15¾ inches. Taxonomists, who are meticulous in arranging their reprints as well as their insects, will find difficulty in filing the map with other standard-sized publications.

L. E. Rozeboom

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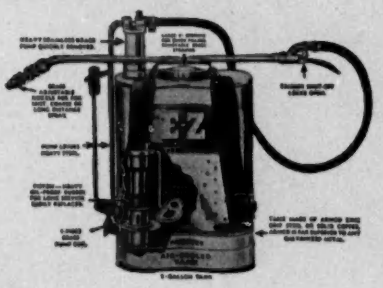

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